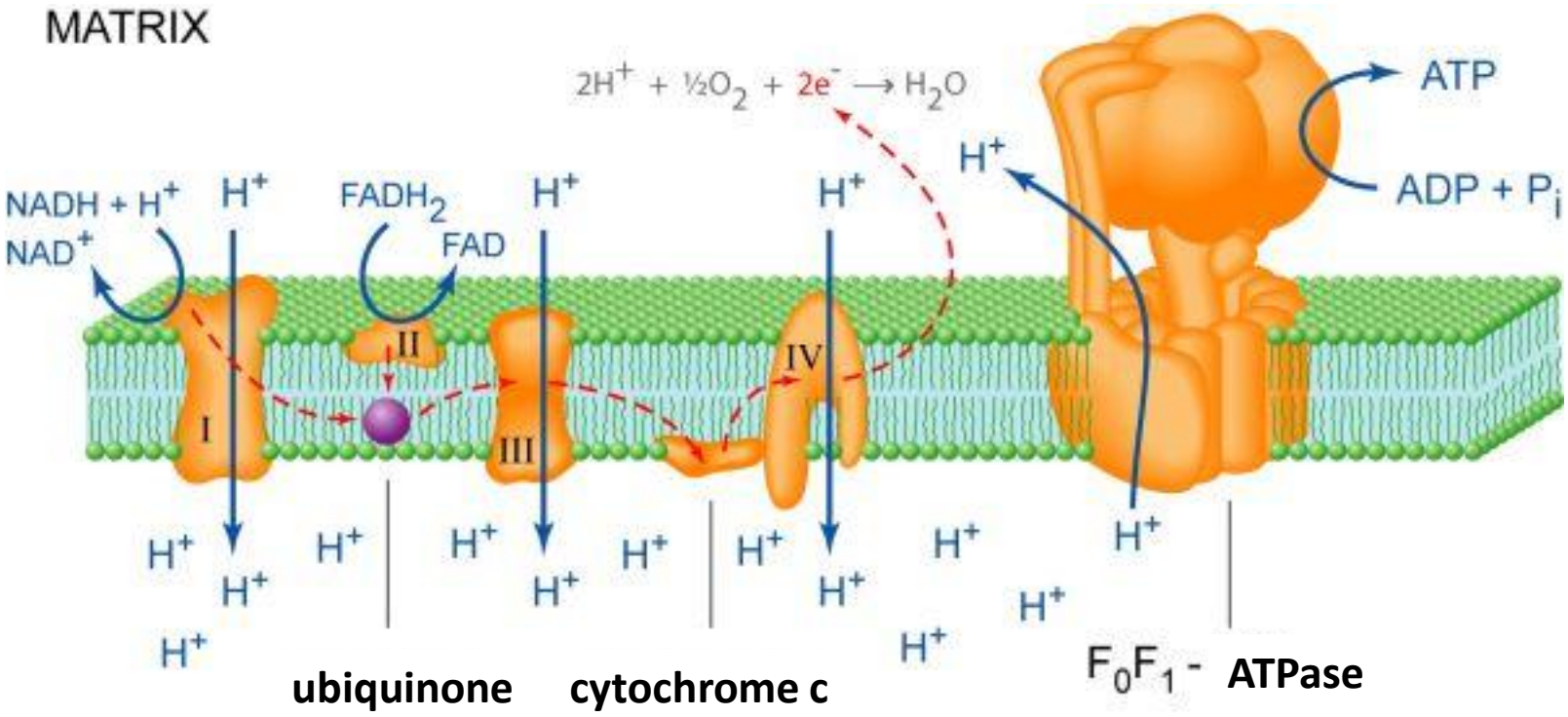
Electron transport chain,

oxidative phosphorylation,

mitochondrial transport systems

JAN ILLNER

Respiratory chain & oxidative phosphorylation



Production of energy in living systems

ATP – source of energy for biochemical reactions

Production of ATP: $ADP + P_i$

1) Substrate phosphorylation – minority part, importance under anaerobic conditions

2) Photosynthetic phosphorylation – chloroplast of green plants, chlorophyll

3) **Oxidative phosphorylation** – dominant mode of ATP production for animal cells

Production of energy in living systems

Utilization of nutrients for the ATP production:

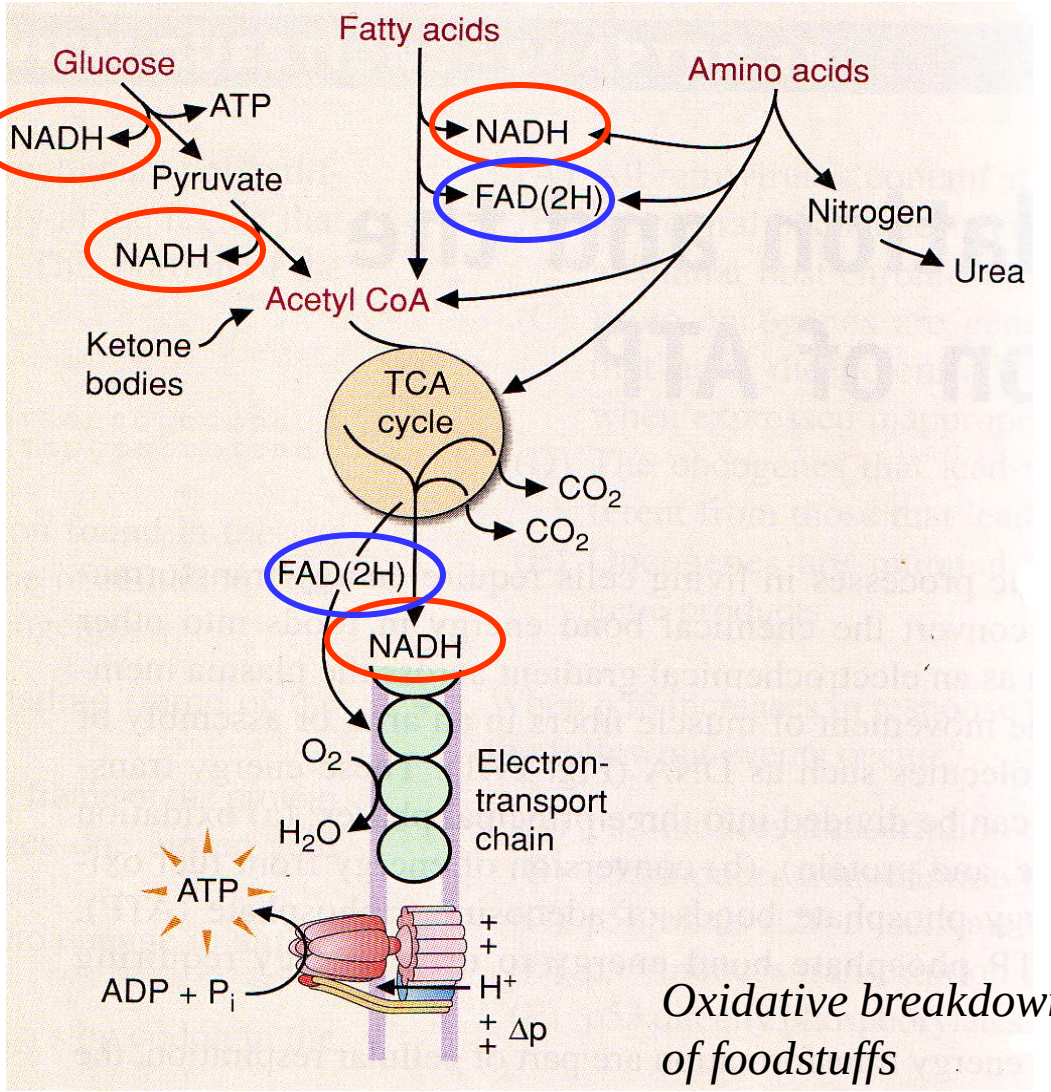
Oxidation by *dehydrogenases*

Production of reduced coenzymes

Transport of reduced equivalents

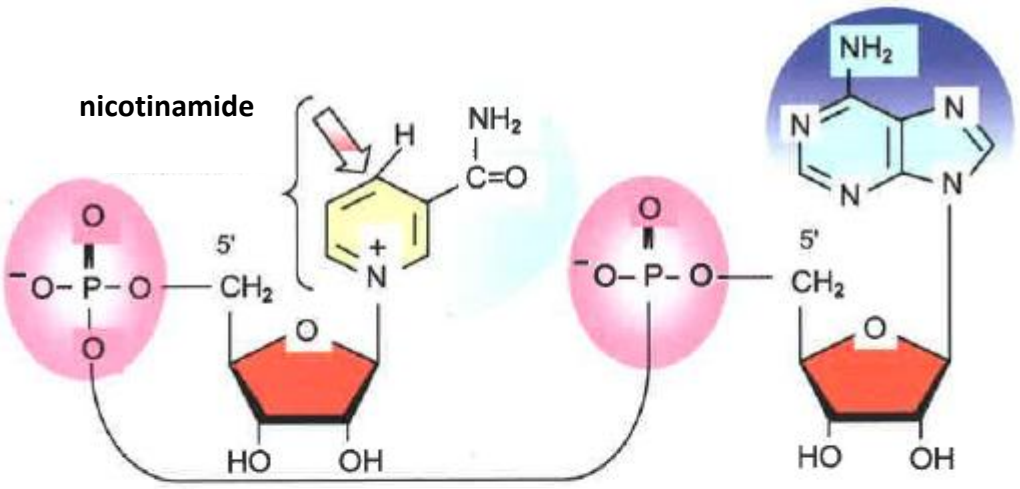
Production of proton gradient

Production of ATP



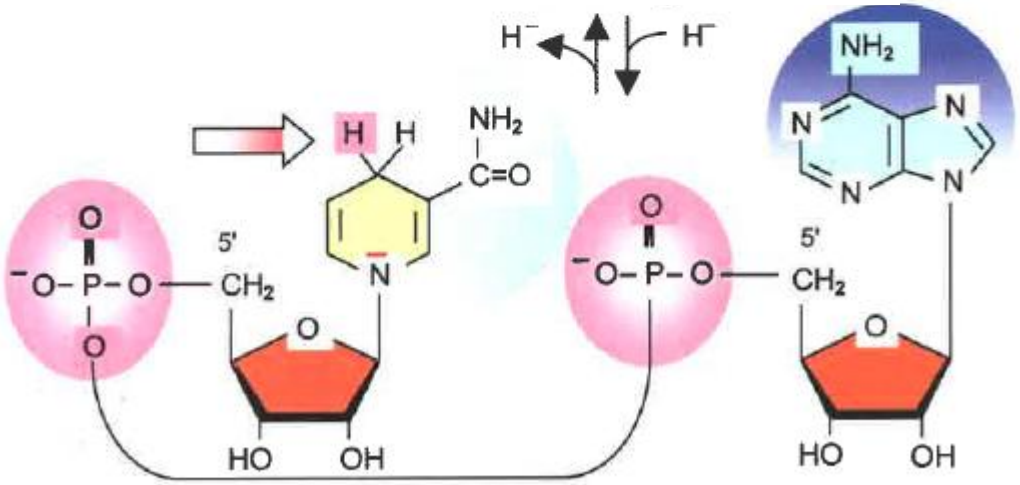
Oxidative breakdown of foodstuffs

Nicotinamide coenzymes



nicotinamide adenine dinucleotide – NAD^+
oxidized form of coenzyme

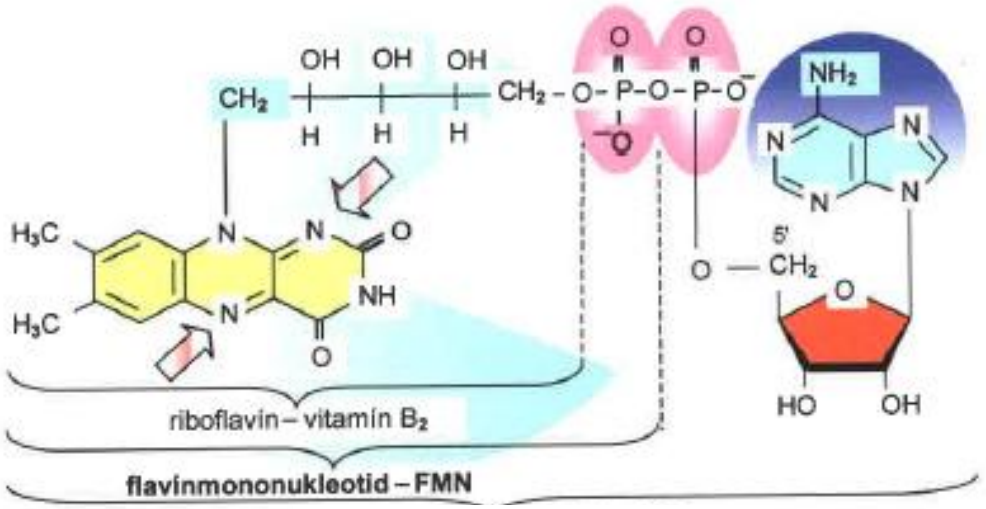
NAD^+



nicotinamide adenine dinucleotide – NADH
reduced form of coenzyme

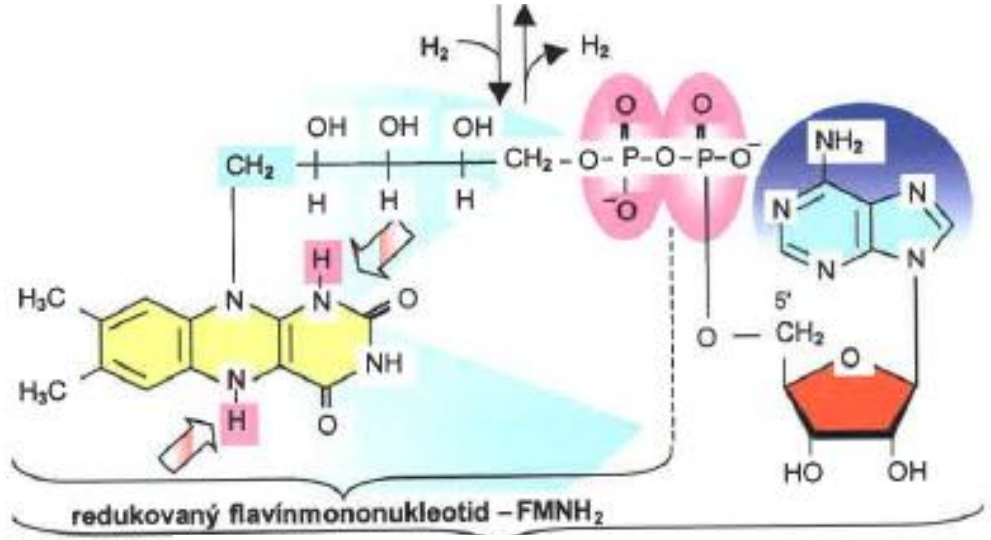
NADH

Flavin coenzymes



flavin adenine dinucleotide – FAD
oxidized form of coenzyme

FAD



flavin adenine dinucleotide – FADH2
reduced form of coenzyme

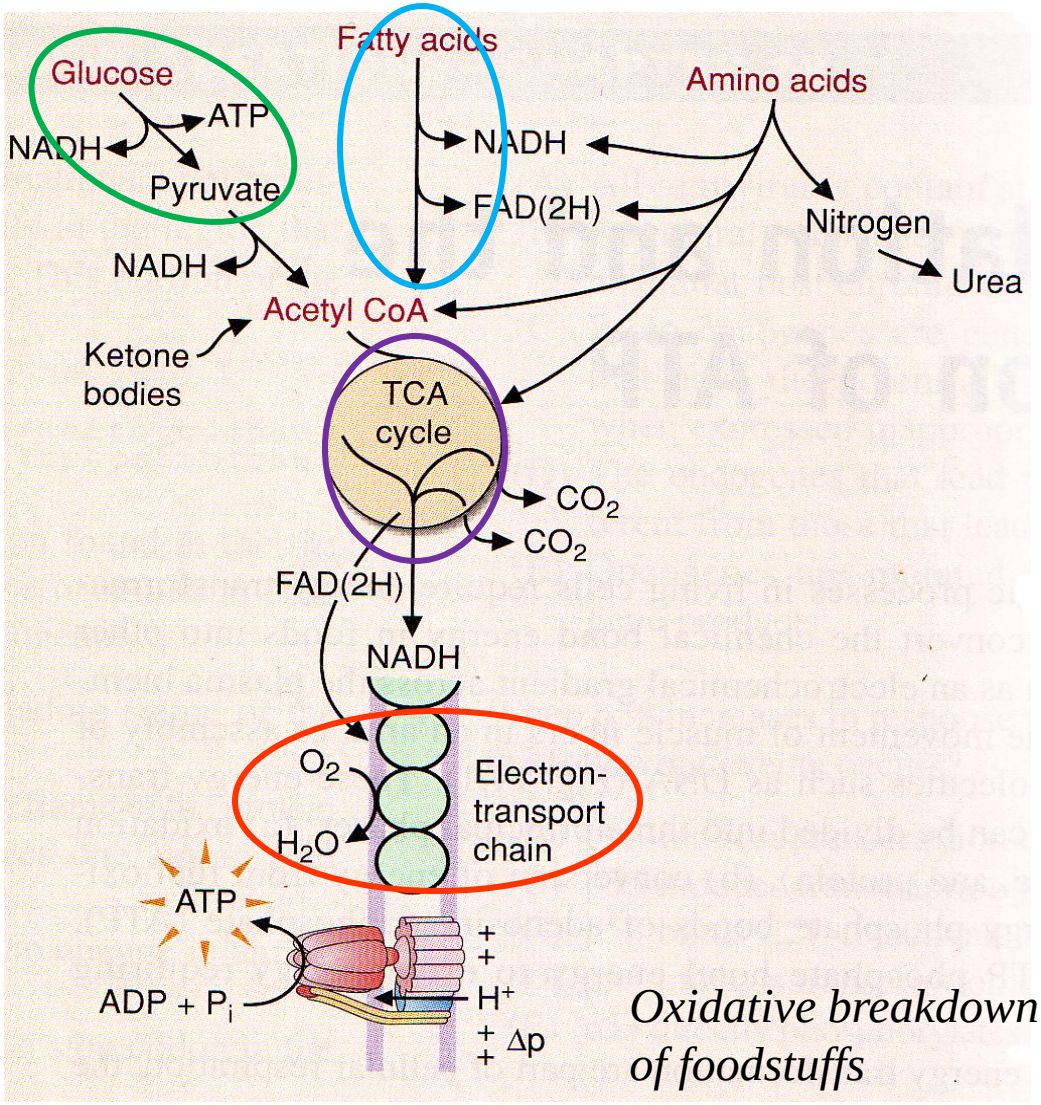
$FADH_2$

Fate of coenzymes

Reduced coenzymes
NADH a $FADH_2$
generated during

TCA cycle
fatty acid oxidation
glycolysis

are **oxidized** by the
electron transport chain



Mitochondria

Inner membrane

large surface ⇨ **cristae**

impermeable, transporters

cardiolipin, enzymes of ETC, *ATP synthase*

Outer membrane

monoaminooxidase (MAO)

permeable through the porin

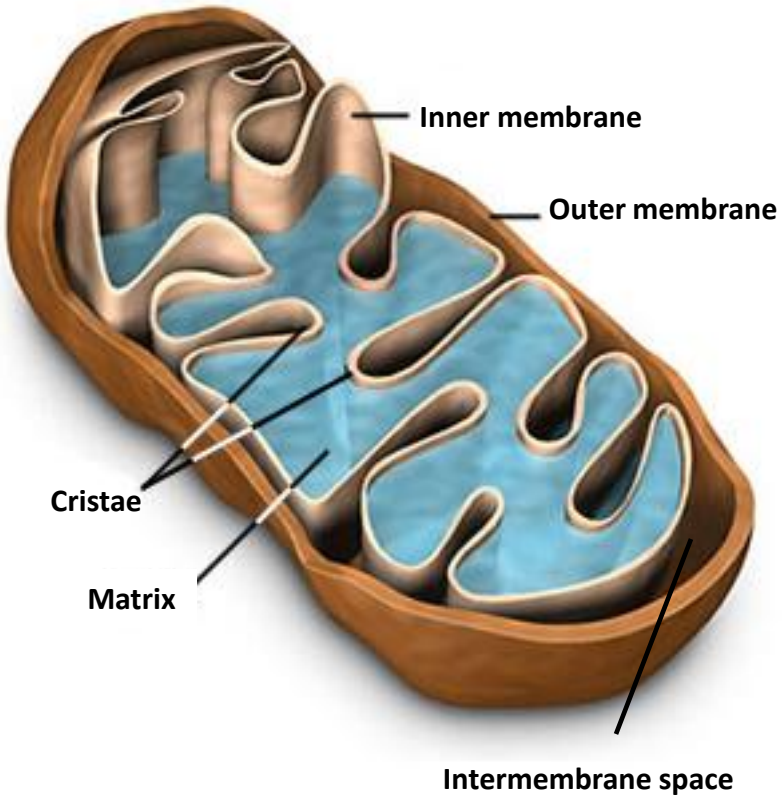
enzymes: *acyl-CoA synthase*, *glycerolphosphate acyltransferase*

Matrix

enzymes of TCA cycle, oxidation of FA, particular synthesis of heme

Intermembrane space

adenylyl kinase, *creatine kinase*



Oxidation – reduction (redox) reactions

$$A_{ox} + B_{red} \longrightarrow A_{red} + B_{ox}$$

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$
$$QH_2 + cyt(Fe^{3+}) \longrightarrow Q + cyt(Fe^{2+})$$
$$cyt(Fe^{2+}) + 2H^+ + 1/2O_2 \longrightarrow cyt(Fe^{3+}) + H_2O$$

E^0 … standard oxidation – reduction **potential**

Redox system	**$E^{0\prime}$ [V]**
$NAD^+/NADH + H^+$	- 0,32
pyruvate/lactate	- 0,19
oxaloacetate/malate	- 0,17
$FAD/FADH_2$	- 0,12
$2H^+/H_2$ (pH = 0)	0
fumarate/succinate	+ 0,03
ubiquinone ox/red	+ 0,10
cytochrome c (Fe^{3+}/Fe^{2+})	+ 0,23
cytochrome a_3 (Fe^{3+}/Fe^{2+}, Cu^{2+}/Cu^+)	+ 0,39
$1/2\ O_2/H_2O$	+ 0,81

Respiratory chain (ETC)

The system of electron carriers transfers **electrons** from reduced coenzymes to final acceptor **O_2**

parts of respiratory chain:

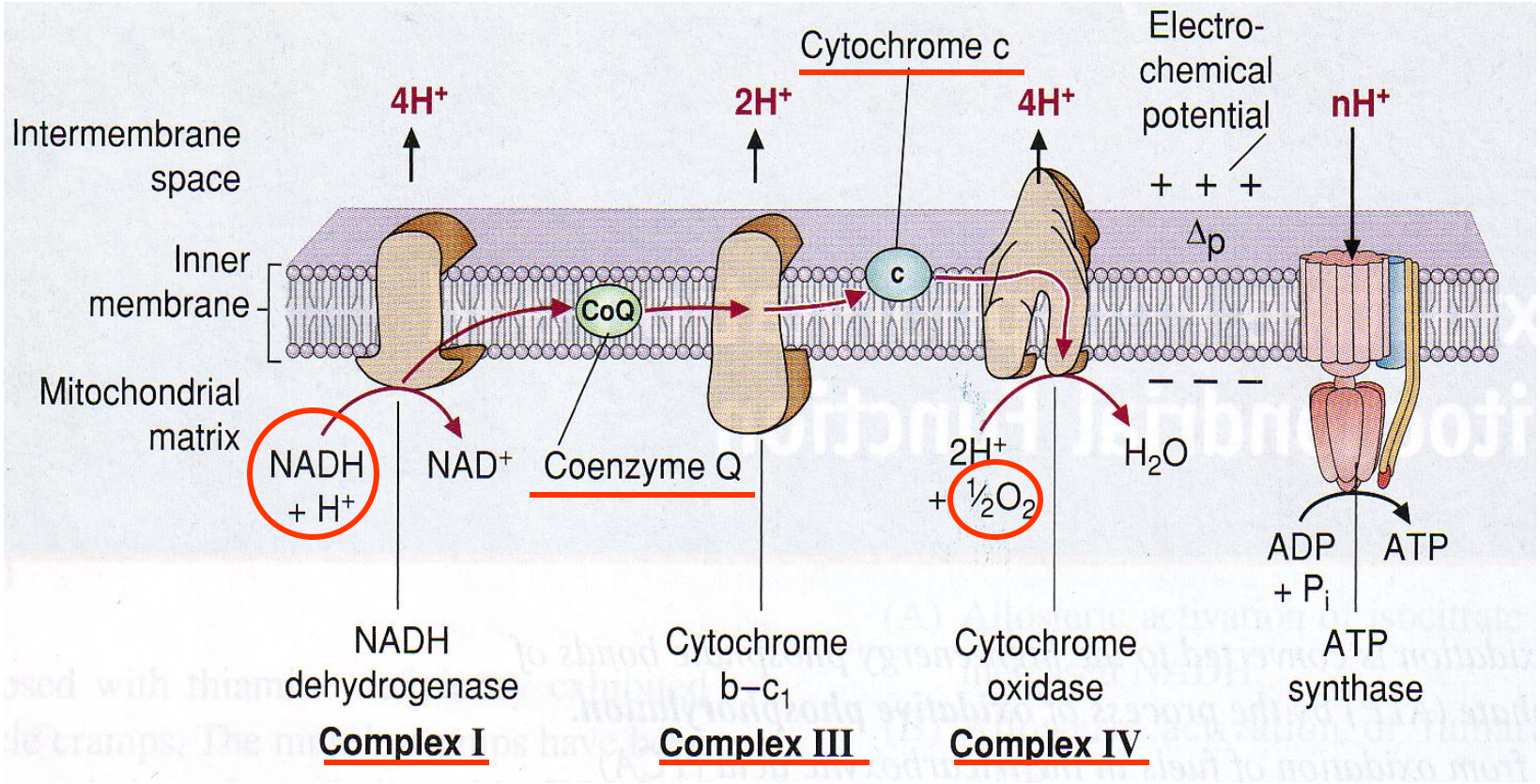
- **4** large enzyme lipoprotein **Green complexes** ⇨ fixed in membrane
- **coenzyme Q** (ubiquinone, CoQ) ⇨ mobile molecules
- **cytochrome c**

electron carriers (ETC complexes) are arranged in order of **increasing** redox potential

= from the most **negative** ($NADH/NAD^+$) to the most **positive** ($1/2\ O_2/H_2O$) redox potential

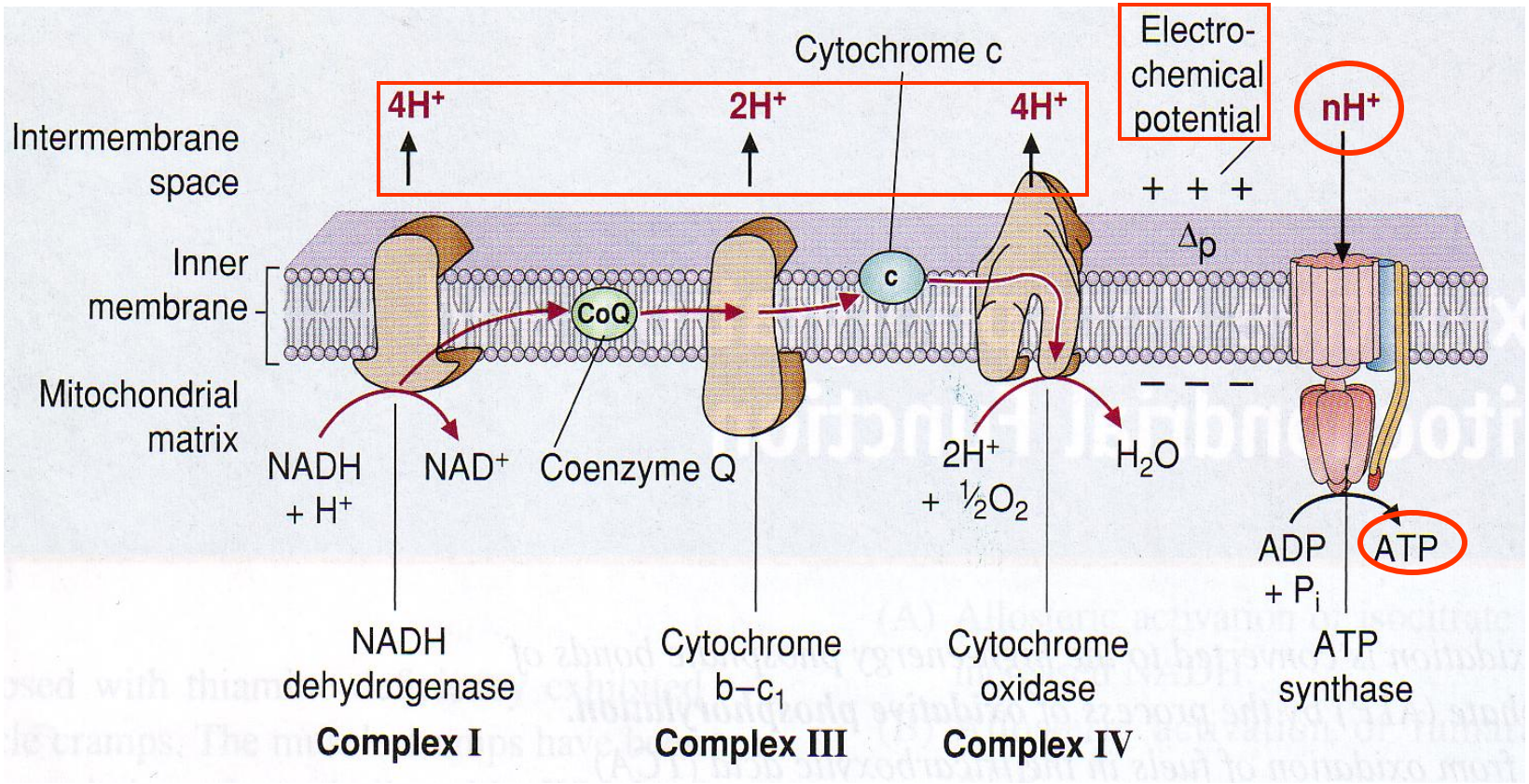
Increasing affinity of electrons, which drives the flow of electron in this direction!

Respiratory chain (ETC)



electrons are transferred from one complex to another in steps to final acceptor oxygen

Respiratory chain (ETC)



during redox reaction the energy is released

⇩

pumping **H⁺** from matrix into <u>intermembrane space</u>

electrochemical gradient

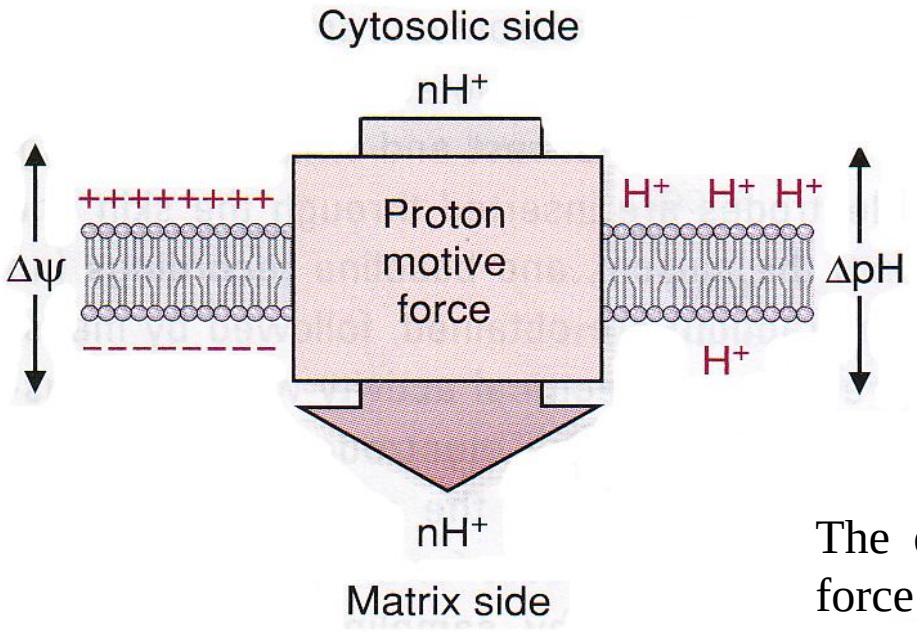
proton gradient

⇩

energy for **ATP synthesis**

Electrochemical potential gradient

= proton-motive force
electric + chemical potential



ΔpH = 0.75-1.0 pH units

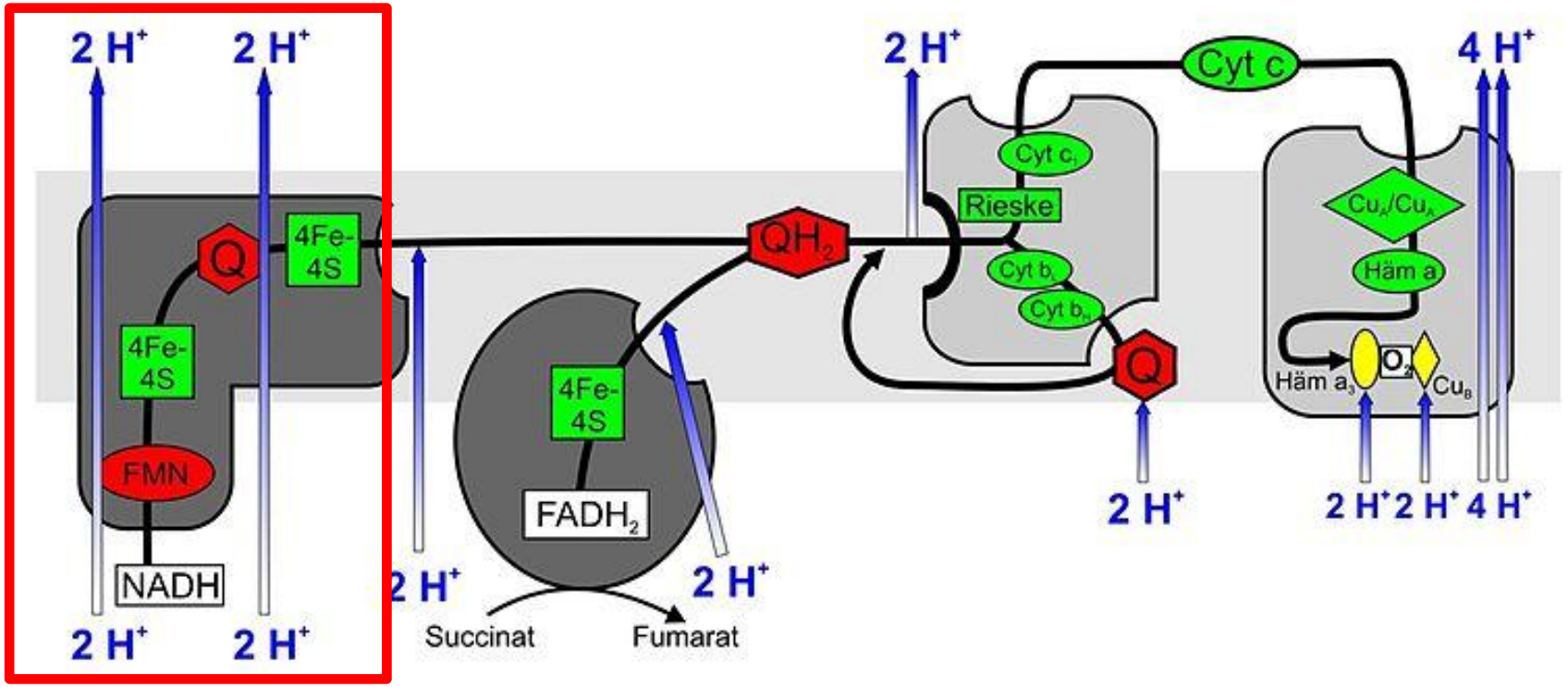
ΔΨ = 0.15-2.0 V

Mitchell s chemiosmotic theory

- mechanisms of electron transfer
- production of proton gradient
- production of ATP

The energy stored in proton-motive force drives the synthesis of ATP by movement of protons down the electrochemical gradient through the ATP-*synthase*

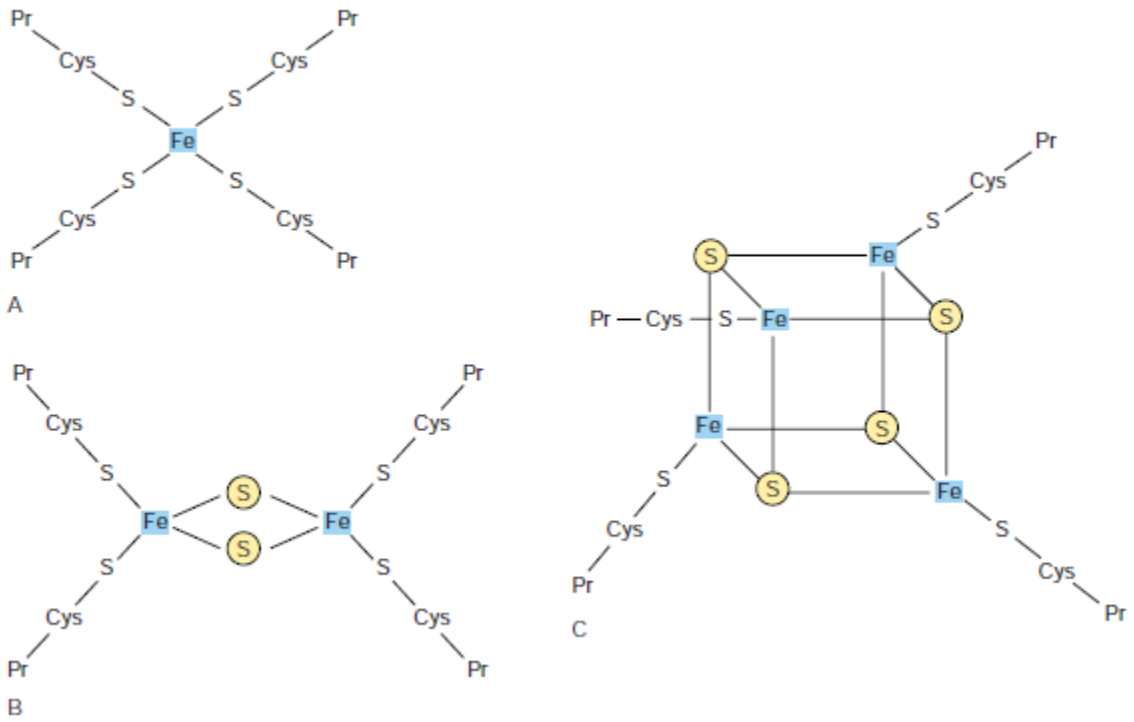
Complex I *NADH: ubiquinone-oxidoreductase*
NADH dehydrogenase



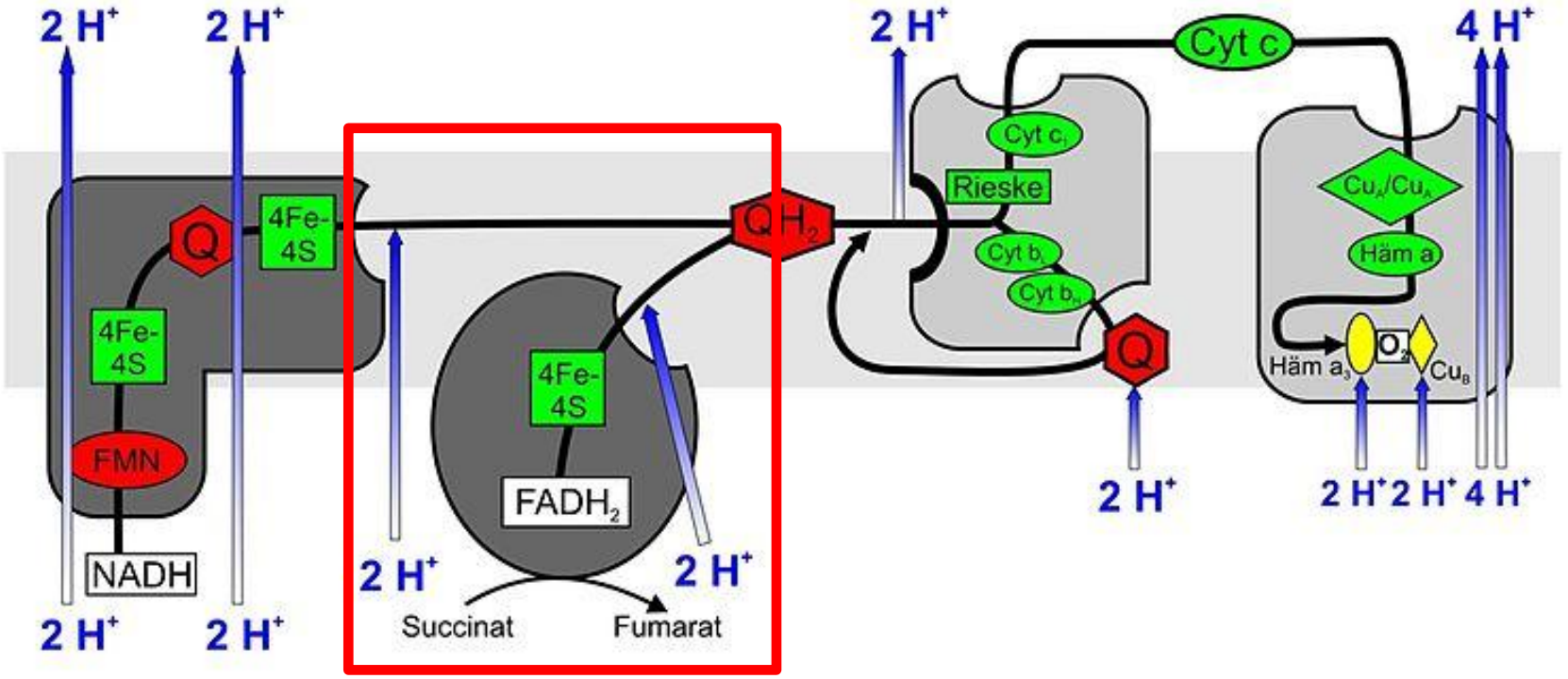
transfer of 2 electrons from NADH to Q associated with transfer of 4 protons through membrane

Iron-sulfur (Fe-S) proteins

proteins contain **non-heme iron** (1, 2 or 4 atoms) a **sulfur**
Fe atoms linked to inorganic S atoms and/or via cysteine –SH groups to the proteins

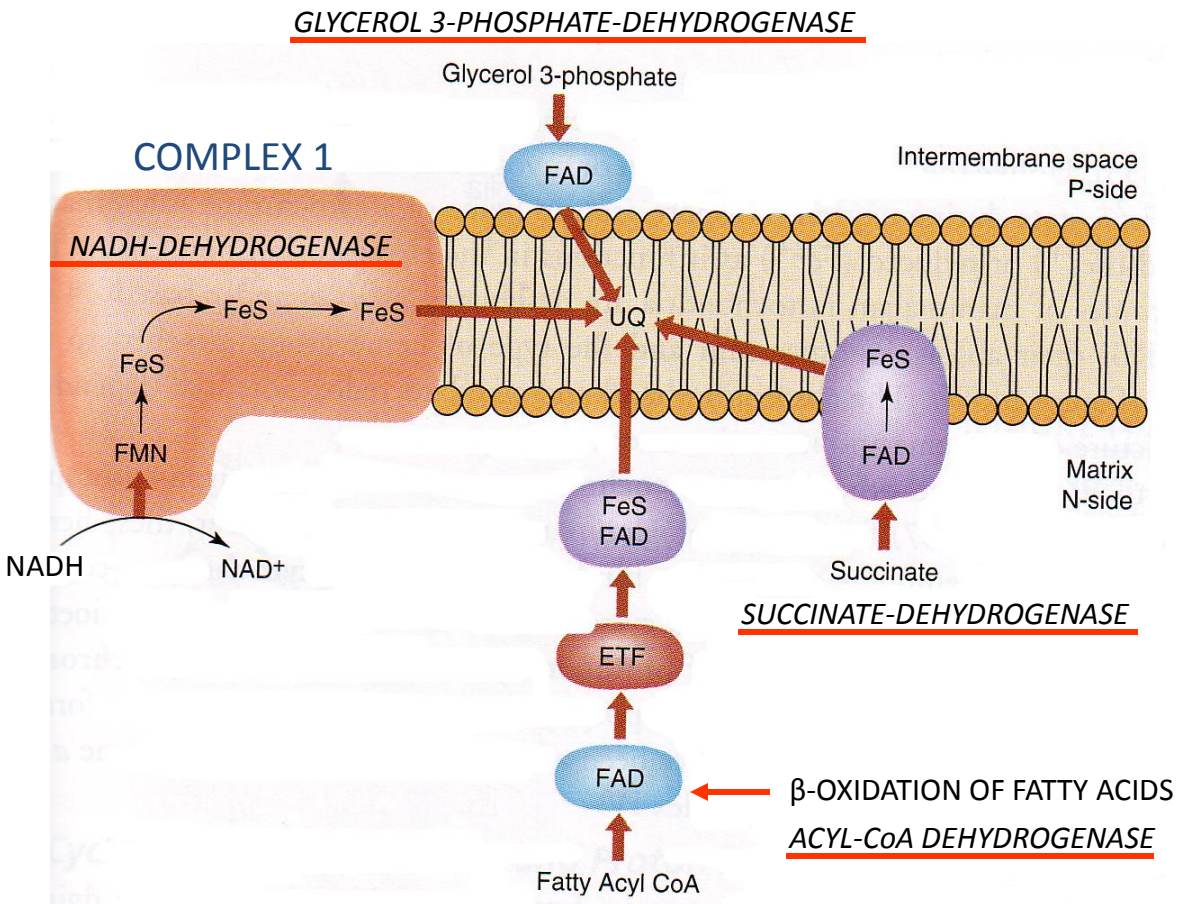


Complex II *succinate: ubiquinone-oxidoreductase succinate dehydrogenase*

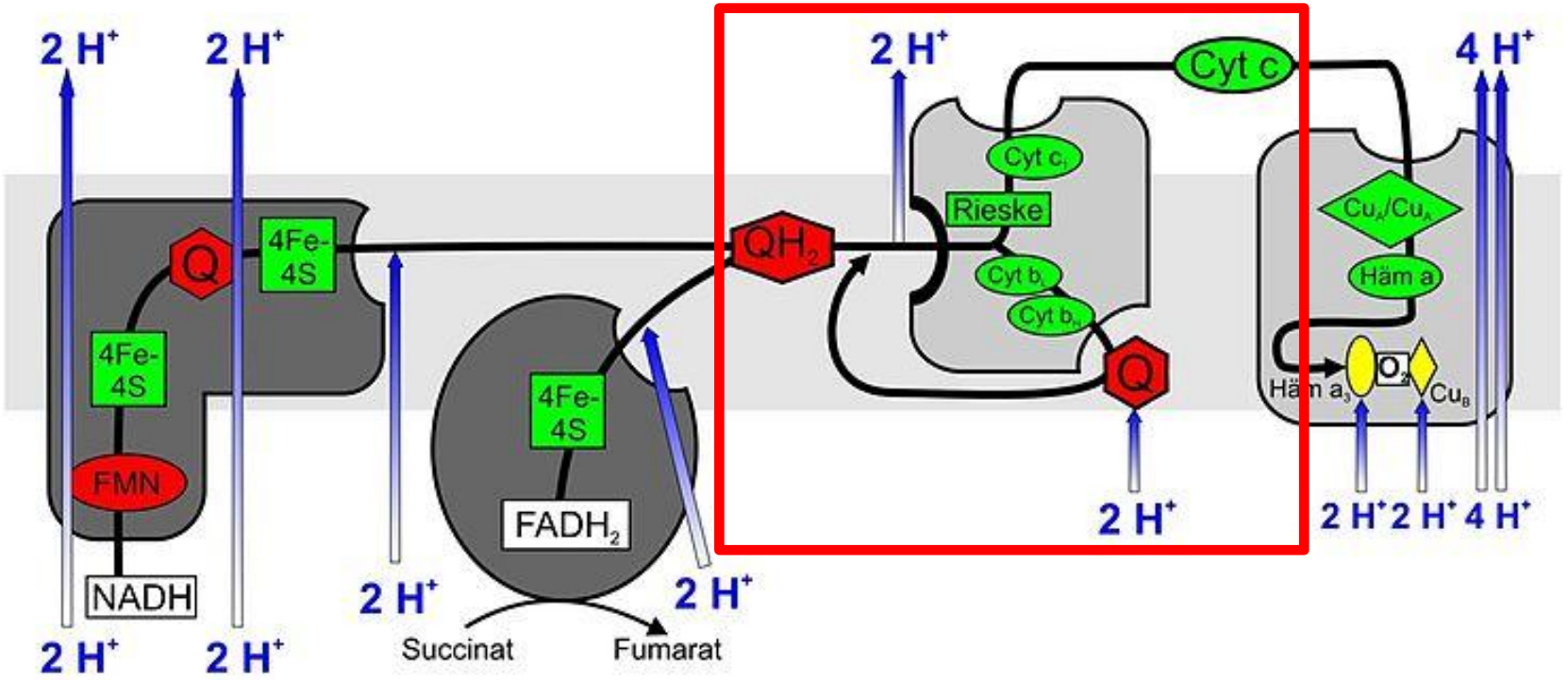


production of $FADH_2$ in TCA cycle and transfer 2 electrons to Q

Oxidation of $FADH_2$ in respiratory chain (ETC)

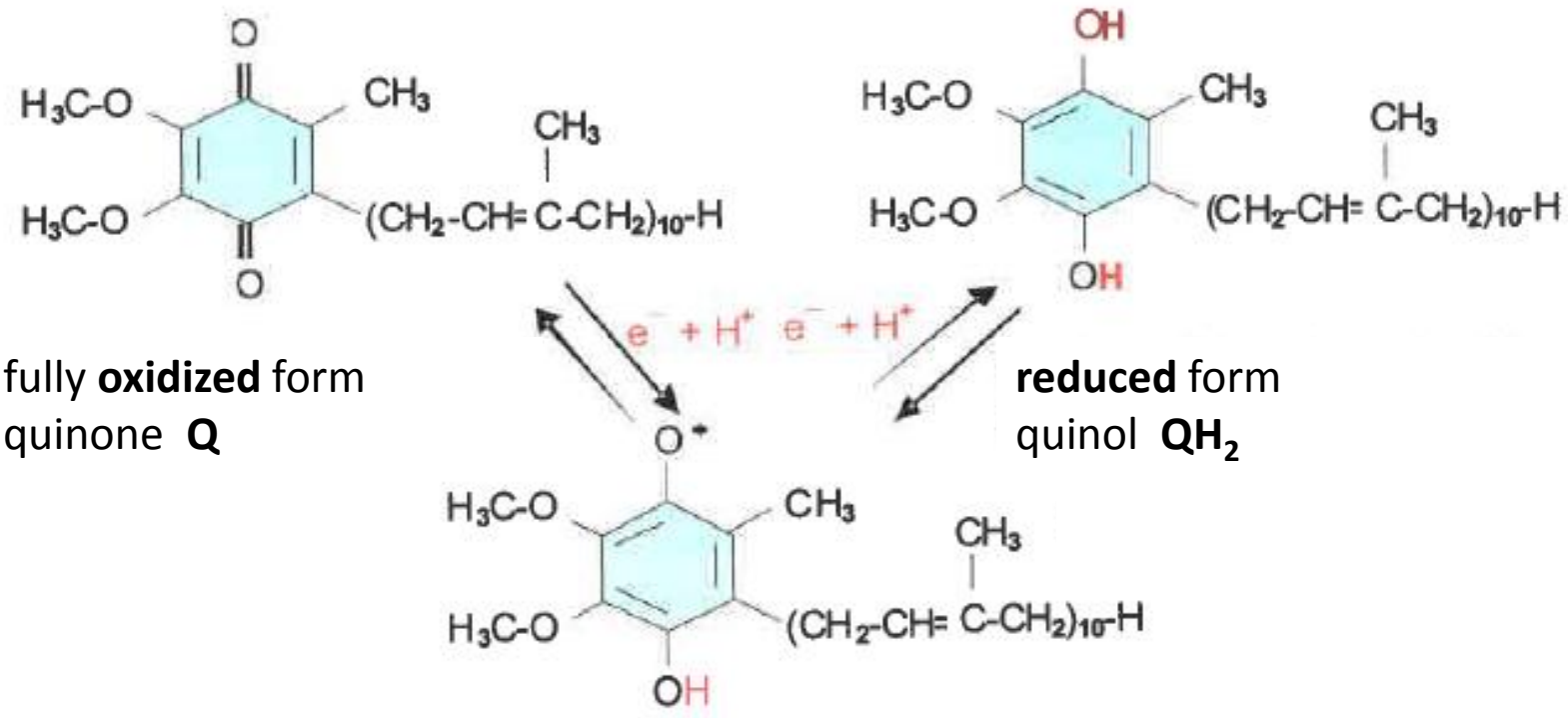


Complex III *ubiquinol: cytochrome c-oxidoreductase cytochrome c reductase*



transfer of 2 electrons from QH_2 to cyt c by cooperation with cyt c_1, b_L, b_H and Rieske s FeS

Forms of coenzyme Q

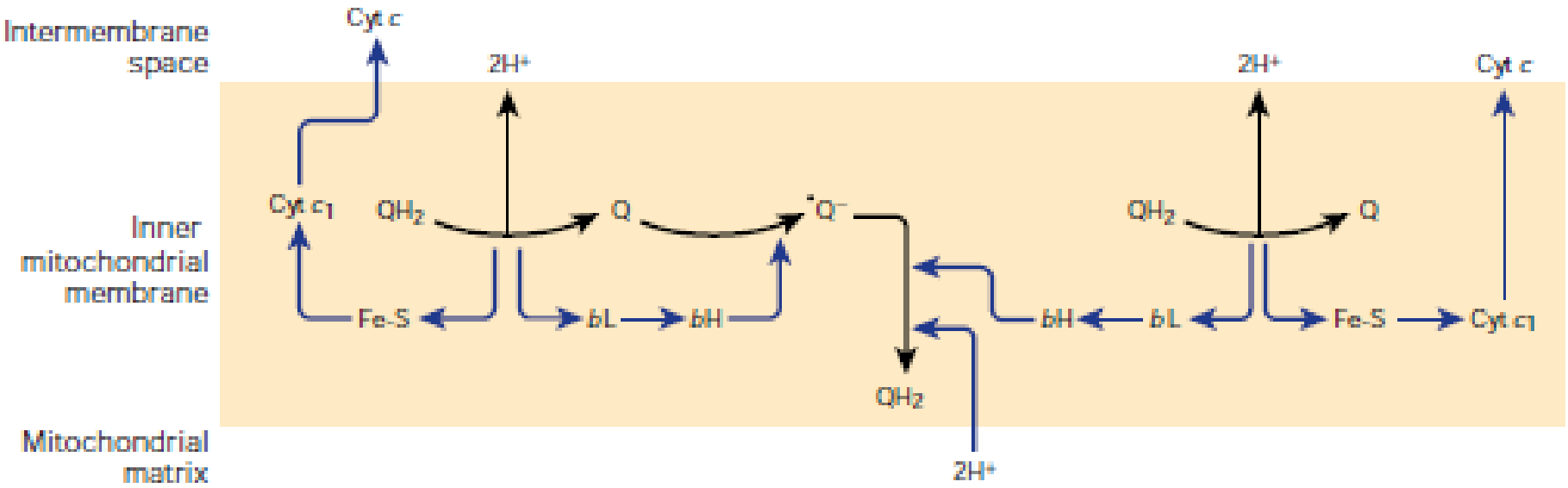


fully **oxidized** form
quinone **Q**

reduced form
quinol **QH_2**

semiquinone form **$Q^{\bullet}$**
free radical

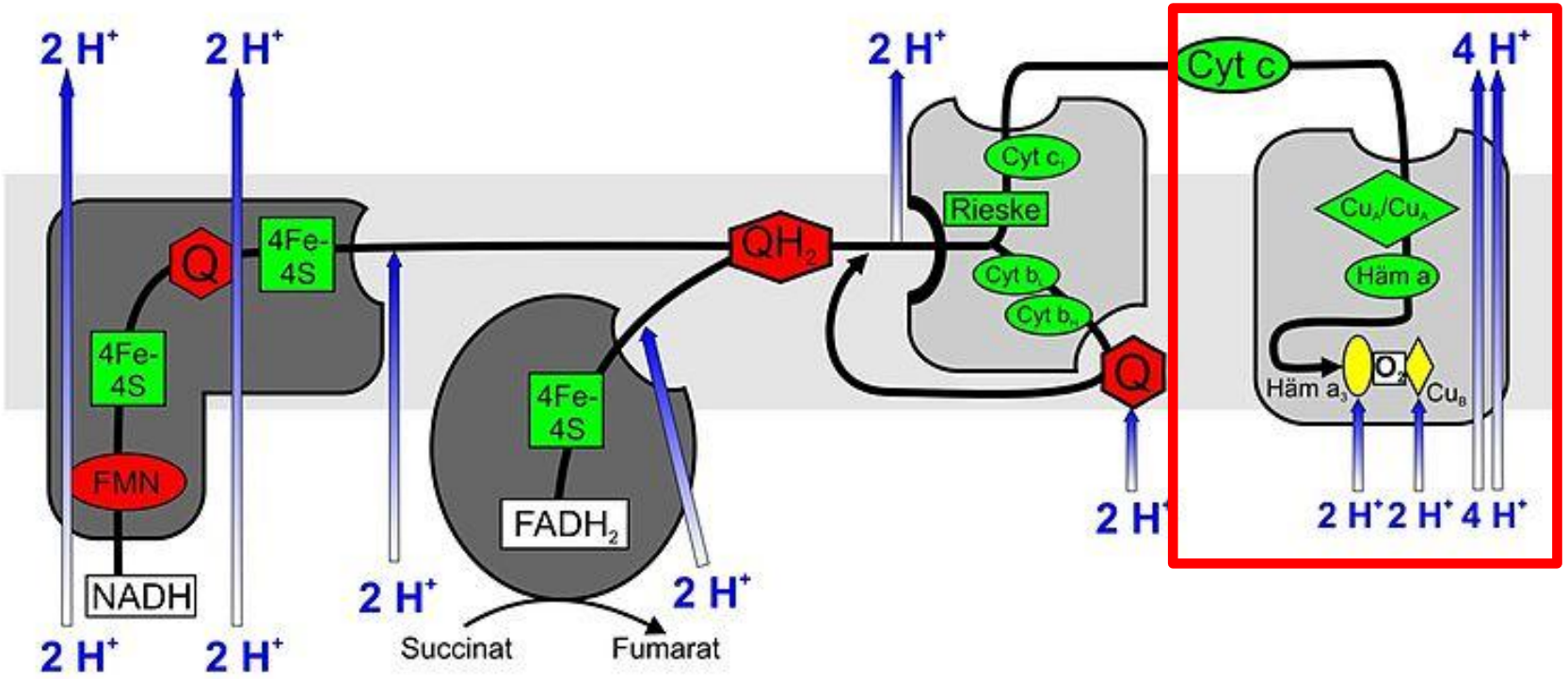
Cycle of coenzyme Q



rotation of 1 cycle leads to

- **oxidation of 2 molecules of QH_2** and **4 H^+ are released** into intermembrane space
- **reduction of 1 molecule of Q** and **2 H^+ are accepted** from matrix

Complex IV *cytochrome c: oxygen-oxidoreductase cytochrome c oxidase*



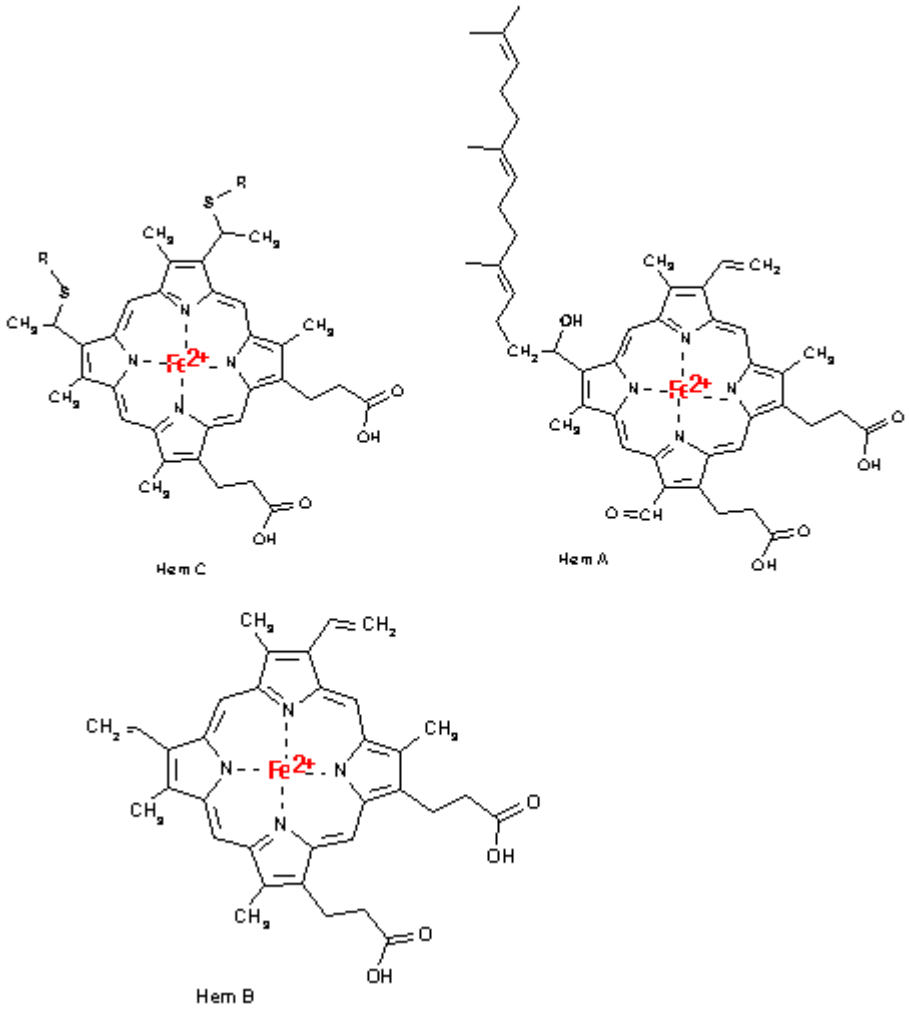
transfer of 4 electrons from cyt c to O_2 by cooperation with hem groups a, a_3 and cooper

8 H^+ is removed from matrix: 4 H^+ are used for production of 2 molecules of water
4 H^+ are transferred into intermembrane space

Cytochrome a, b, c

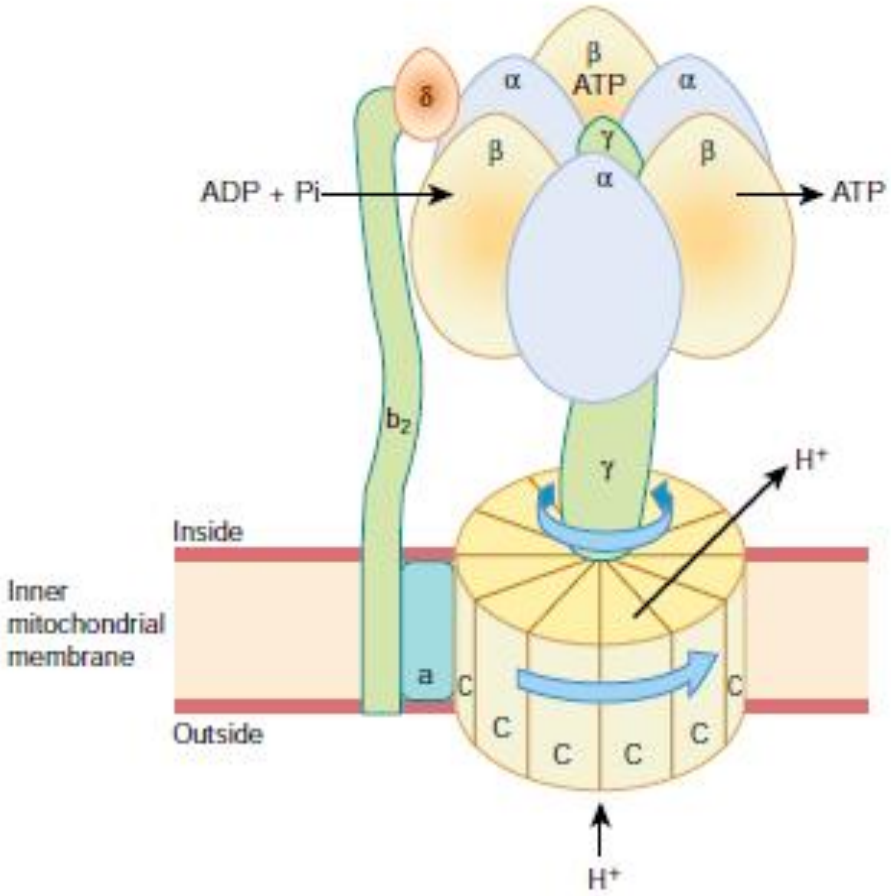
- **heme proteins** contain protoporphyrin IX and Fe atom

- cytochromes have different <u>side chains</u>



„Complex V“ *ATP synthase*

localized in inner membrane, driven by H^+, production of ATP = ADP + P_i



F_1
head

F_0 subunit: proton channel
C protein complex with γ subunit
passage of H^+ = **rotational movement**

F_1 subunit: phosphorylation mechanism
head with three α and three β subunits
connected with membrane, **no rotation**

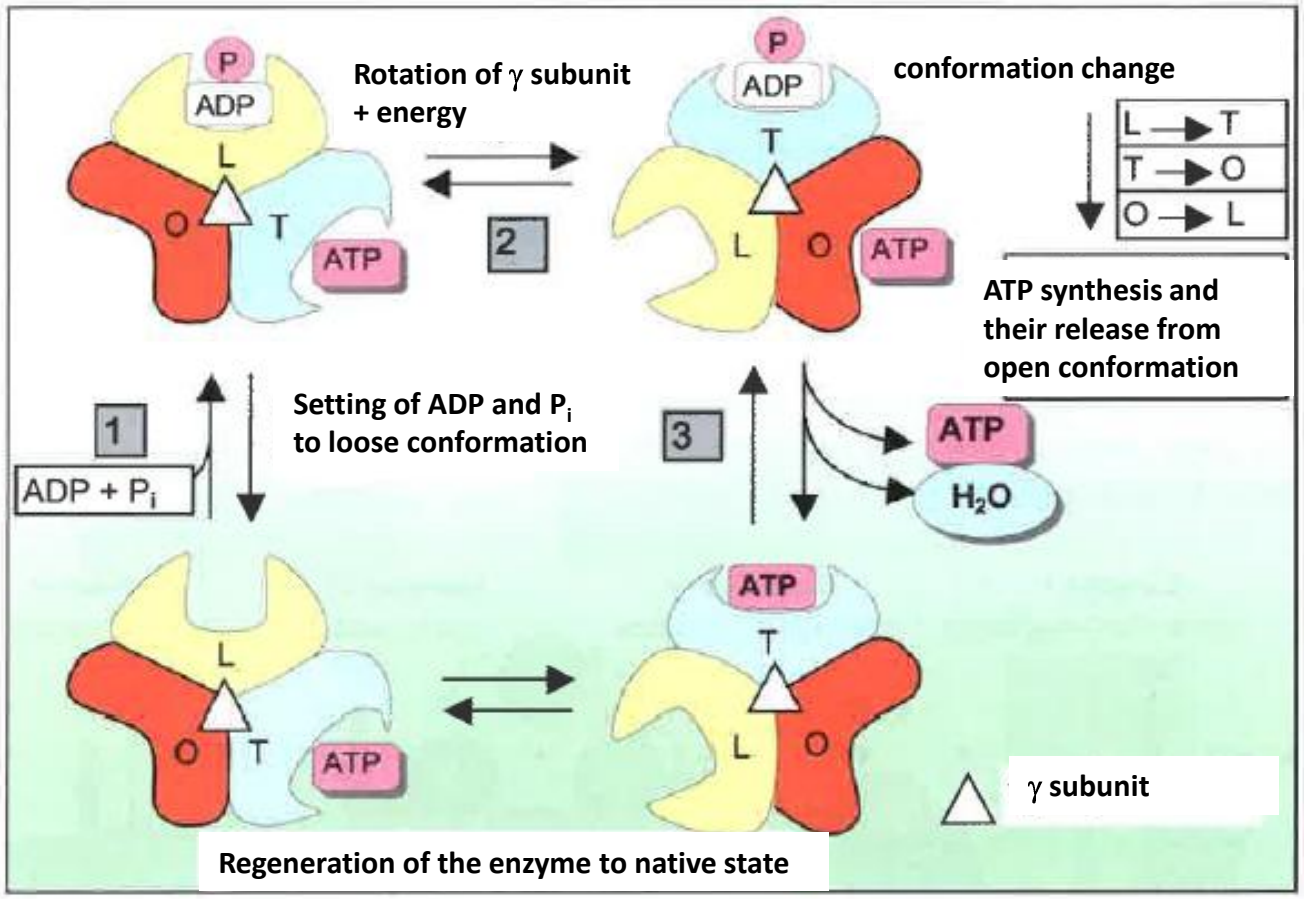
F_0
pore

ATP synthesis

ADP and P_i are transferred to γ subunit, production of ATP
ATP is released after β subunits conformation change

3 conformation of β subunit:

loose (**L**)
tight (**T**)
open (**O**)

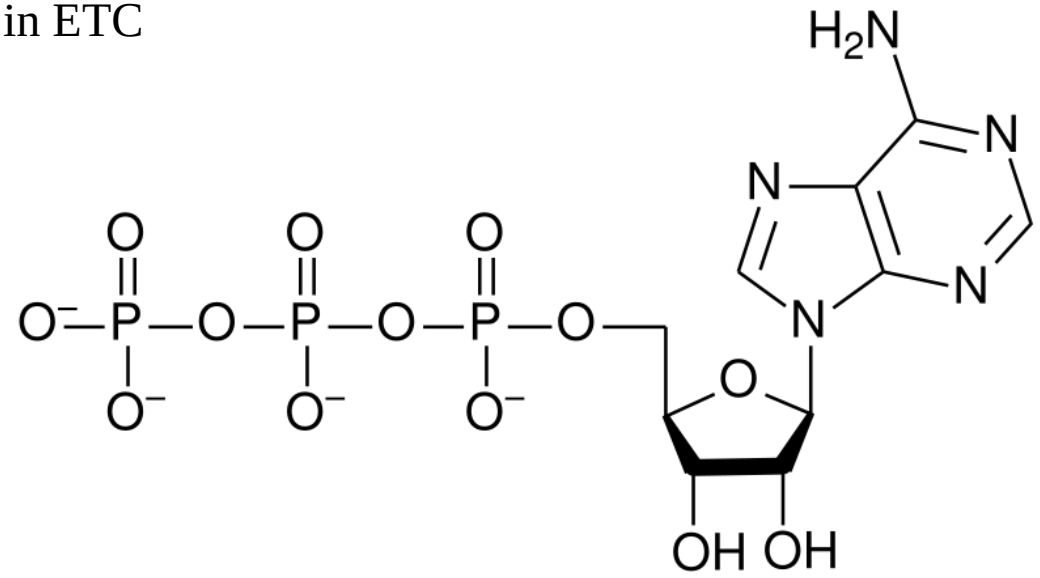


Majority of energy is generated in respiratory chain

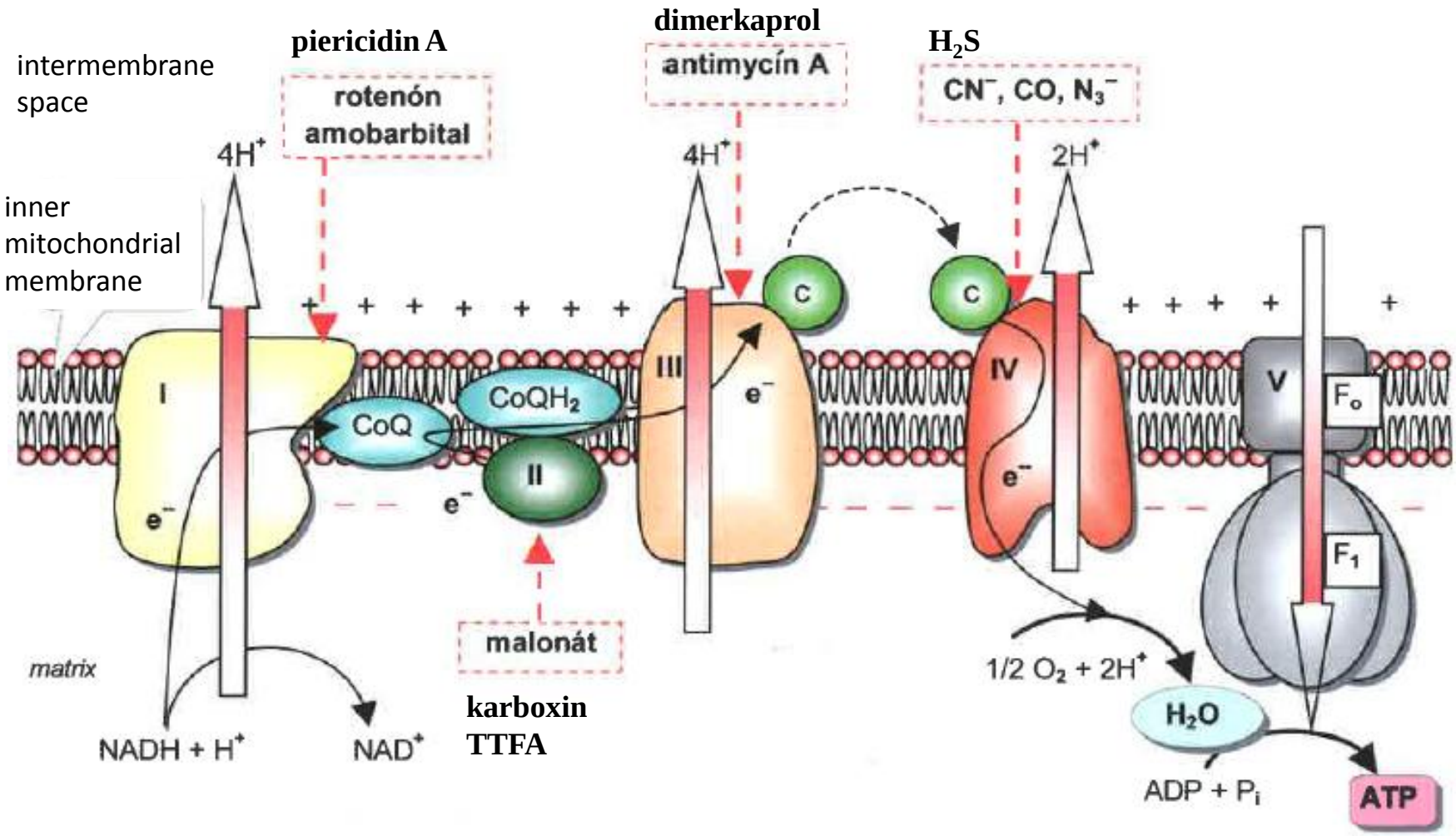
NADH oxidation (2 e^-)……. **2,5 mol of ATP**
1 mol of substrate is oxidized through complexes **I**, III and IV

$FADH_2$ oxidation (2 e^-)…… **1,5 mol of ATP**
1 mol of substrate is oxidized by complexes **II**, III and IV

Overall, 30 – 32 mol of ATP from oxidation of 1 mol of glucose,
26 – 28 mol of ATP is generated in ETC



Inhibitors of respiratory chain

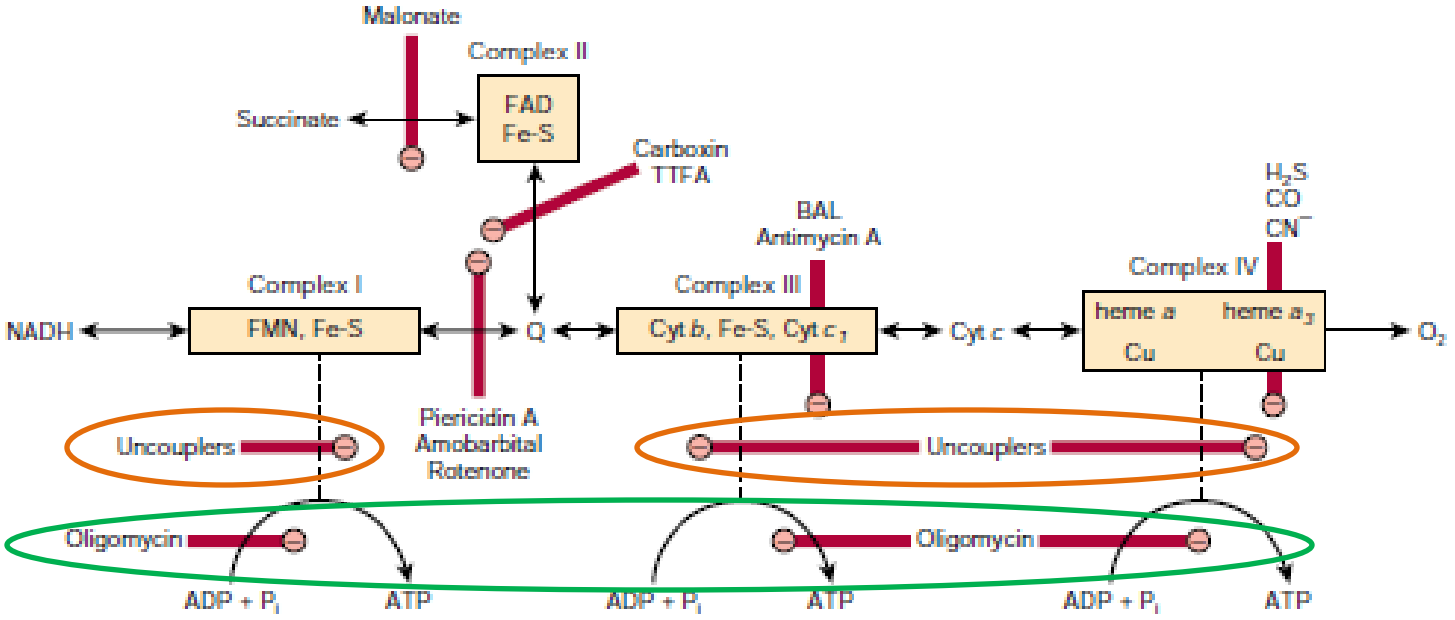


Inhibitors of oxidative phosphorylation

inhibitors – foreign substances that are toxic in case of energy production process
inhibitory action is specific for specific place

antraktylosid – inhibits transporter of ADP into and ATP out of the mitochondrion

oligomycin – completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP *synthase*

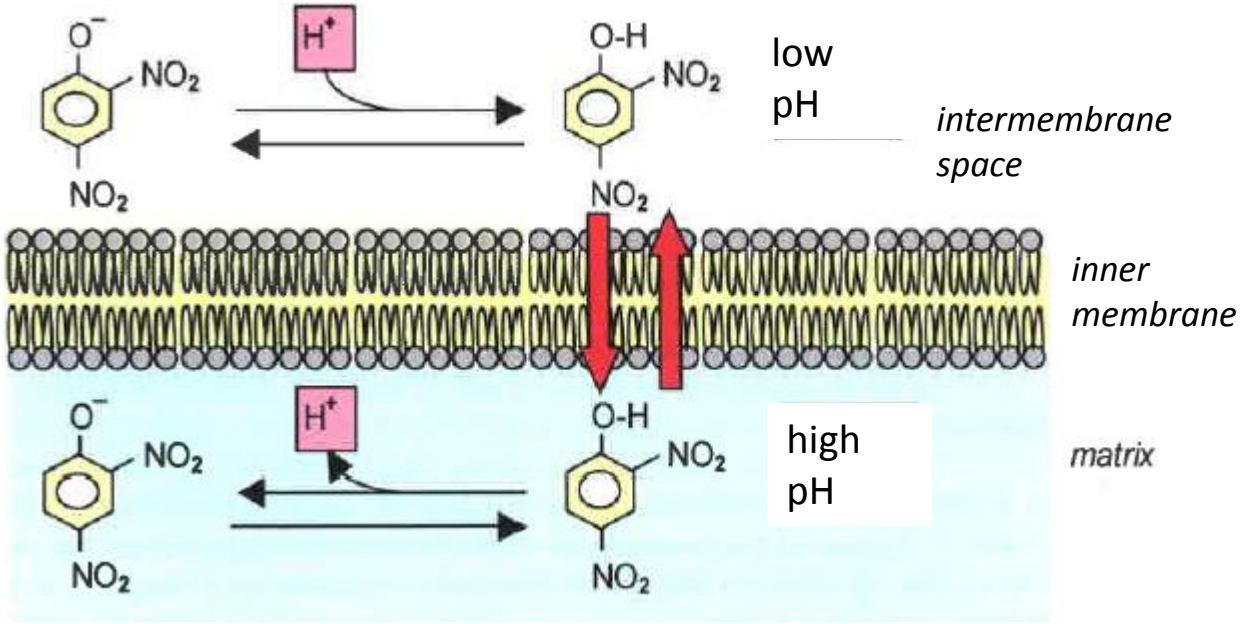


Uncouplers of oxidative phosphorylation

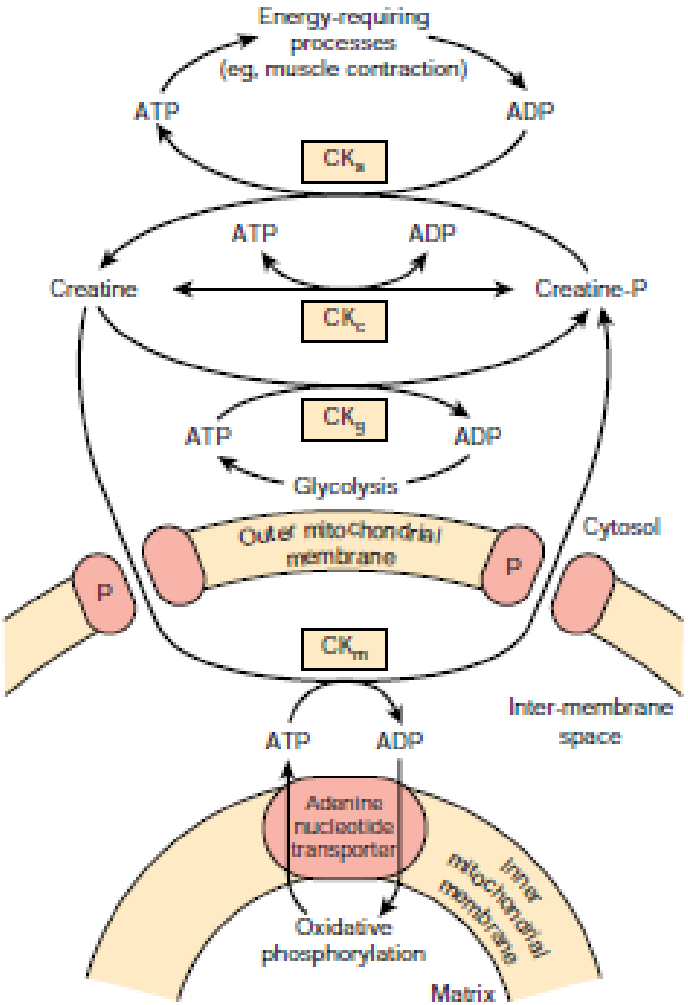
uncouplers – dissociate oxidation in the respiratory chain from phosphorylation
in vivo **toxic compounds** causing respiration to become uncontrolled

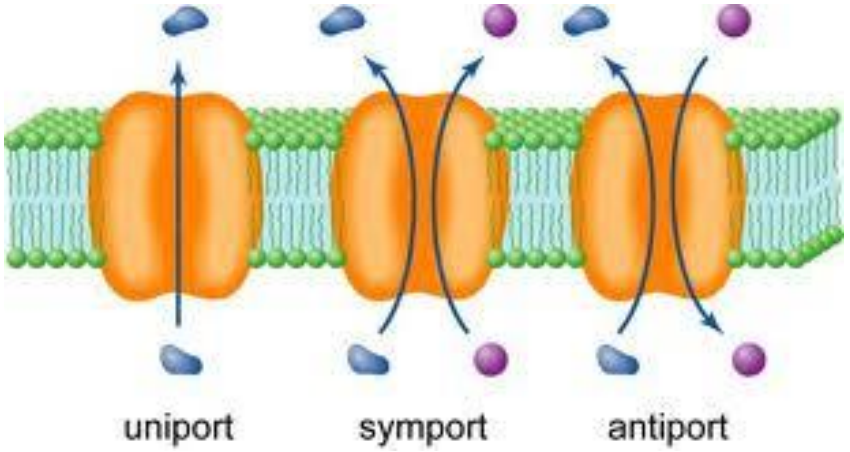
2,4-dinitrophenol – transfers proton through membrane, disrupts proton gradient

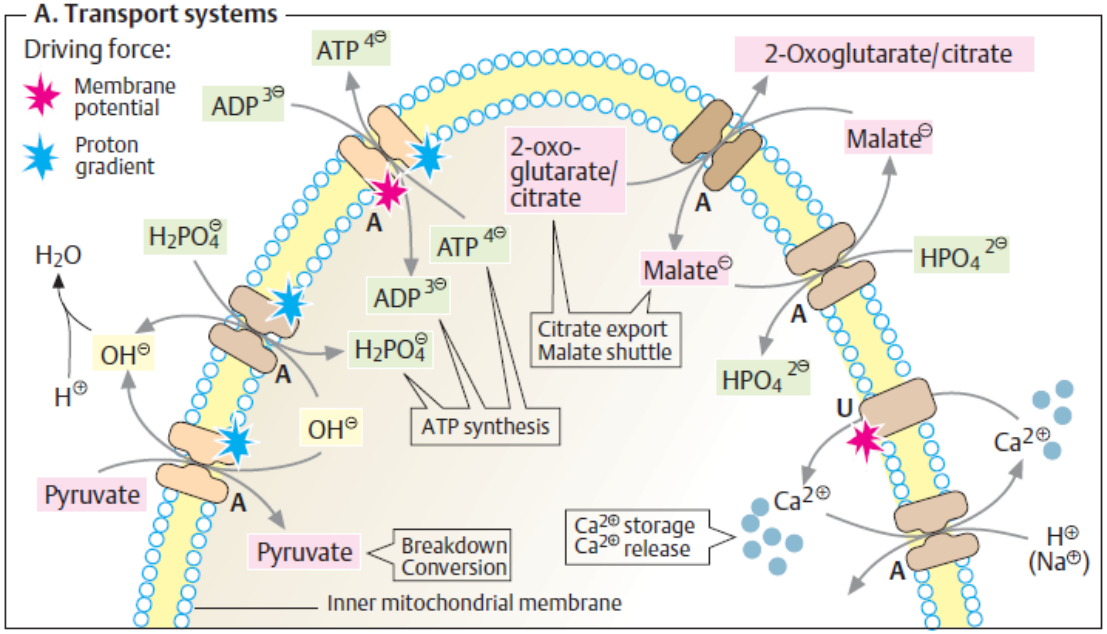
thermogenin – physiological uncoupler in brown adipose tissue



Mitochondrial transport systems







Mitochondrial transporters

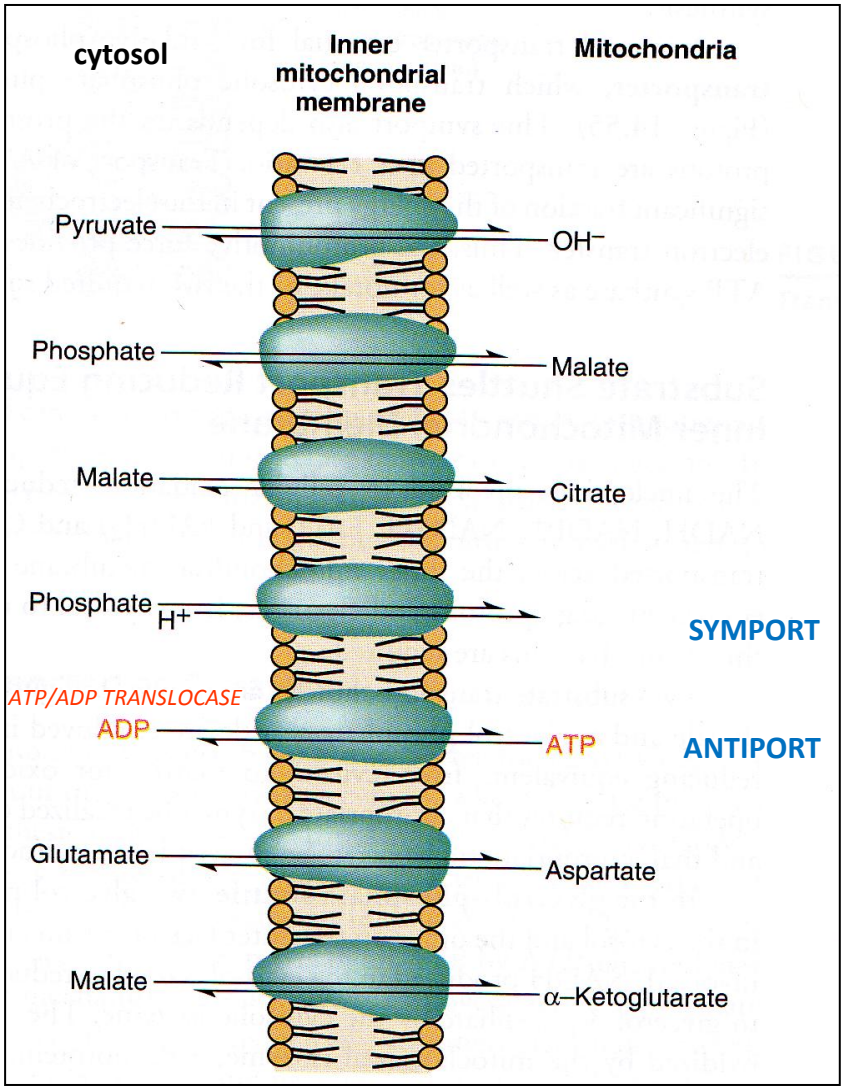
Inner membrane is freely <u>permeable **only**</u> for:

a) small uncharged molecules: H_2O, CO_2, NH_3

b) **mono**carboxylic acids (3-hydroxybutyric, acetic)

Long-chain **fatty** acids – via the **carnitine system**

Di- and **tri**karboxylate anions and **amino** acids – **specific transporters**



Shuttles

Transport systems cytosol – mitochondria

NADH cannot penetrate the mitochondrial membrane

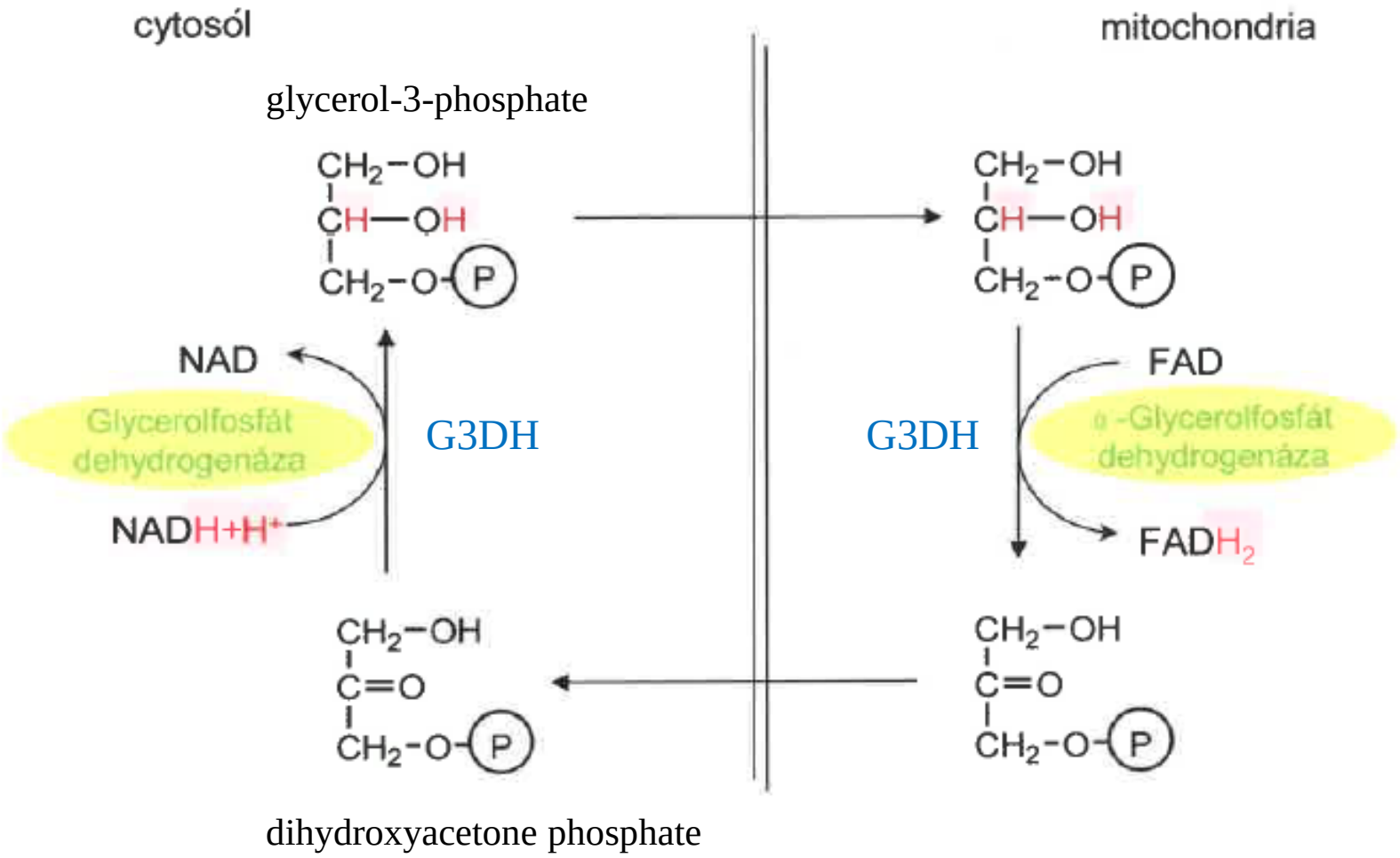
Transfer through membrane requires **substrate pairs**, linked by suitable *dehydrogenases* on each side of membrane

Glycerophosphate shuttle

Malate shuttle

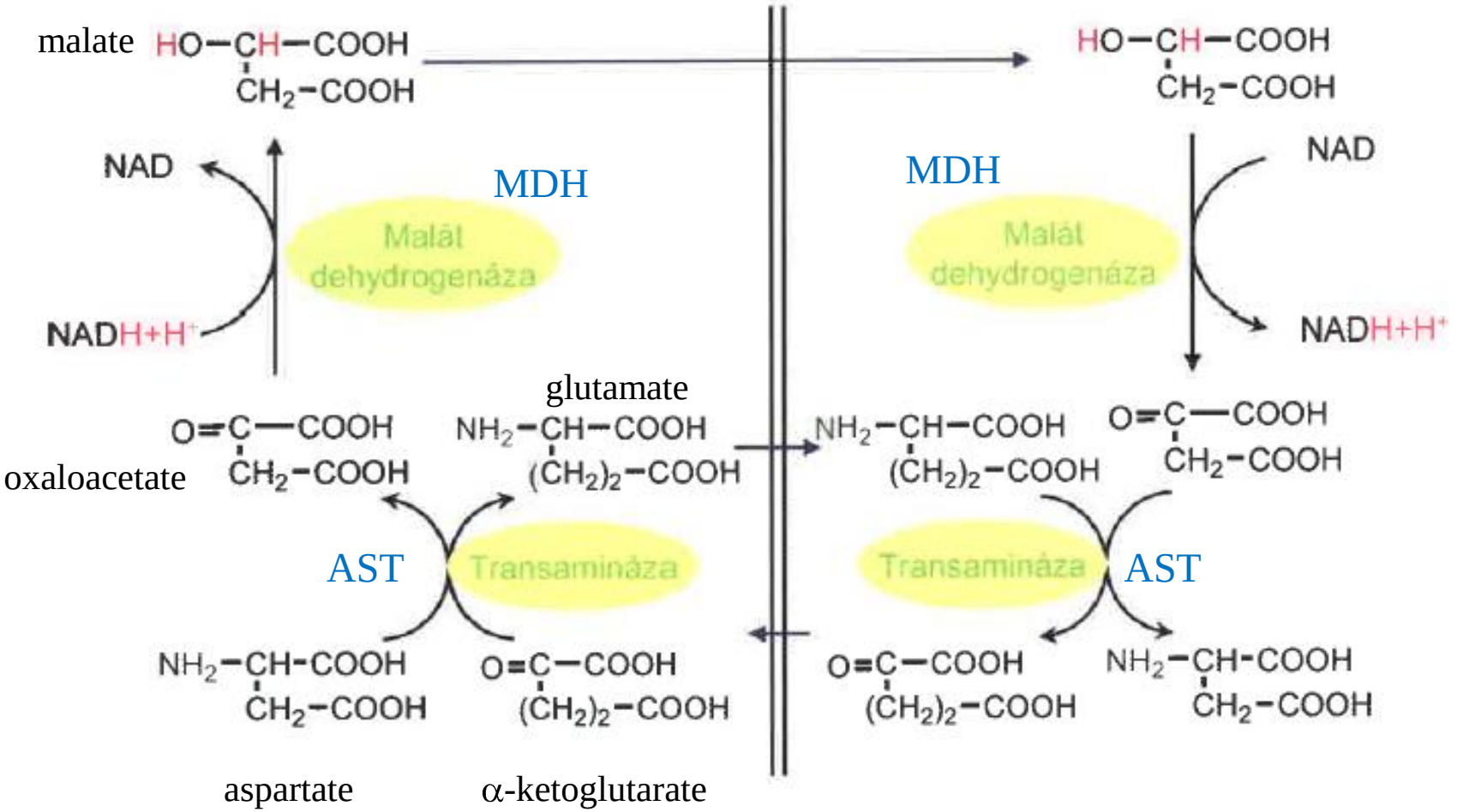
Glycerophosphate shuttle

Enzyme: *glycerol-3-phosphate dehydrogenase* (G3PDH)



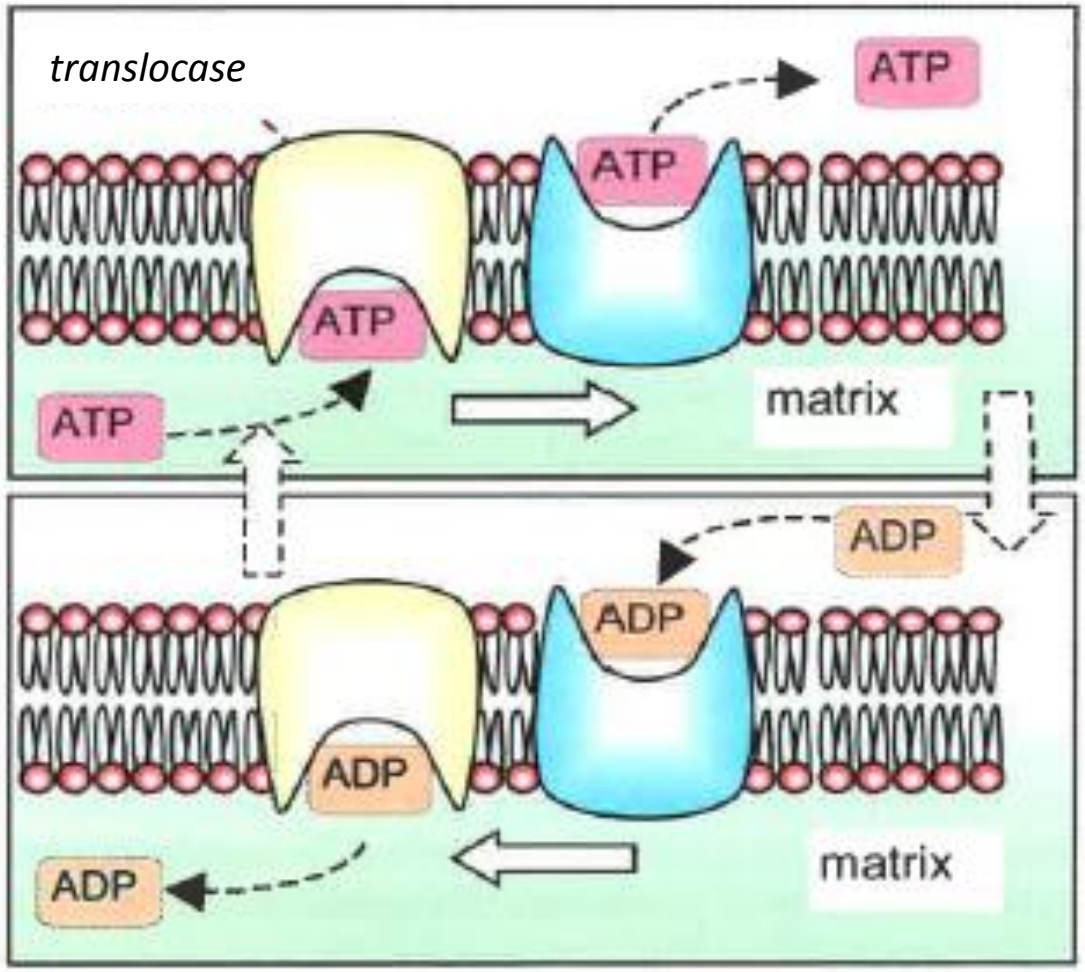
Malate shuttle

Enzymes: *malate dehydrogenase* (MDH), *glutamate oxaloacetate transaminase* (GOT) = *aspartate aminotransferase* (AST)

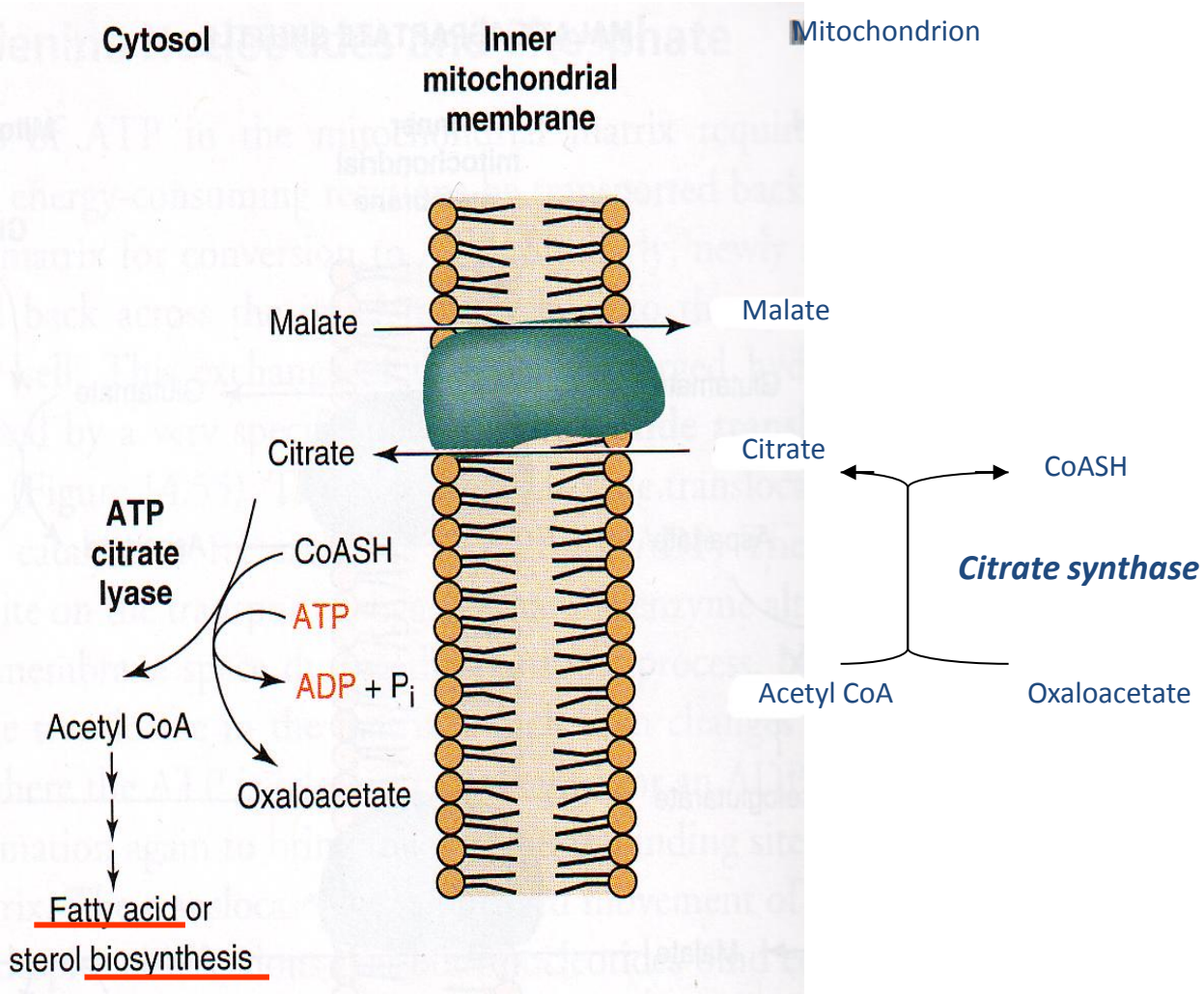


Transfer of ADP/ATP through membrane

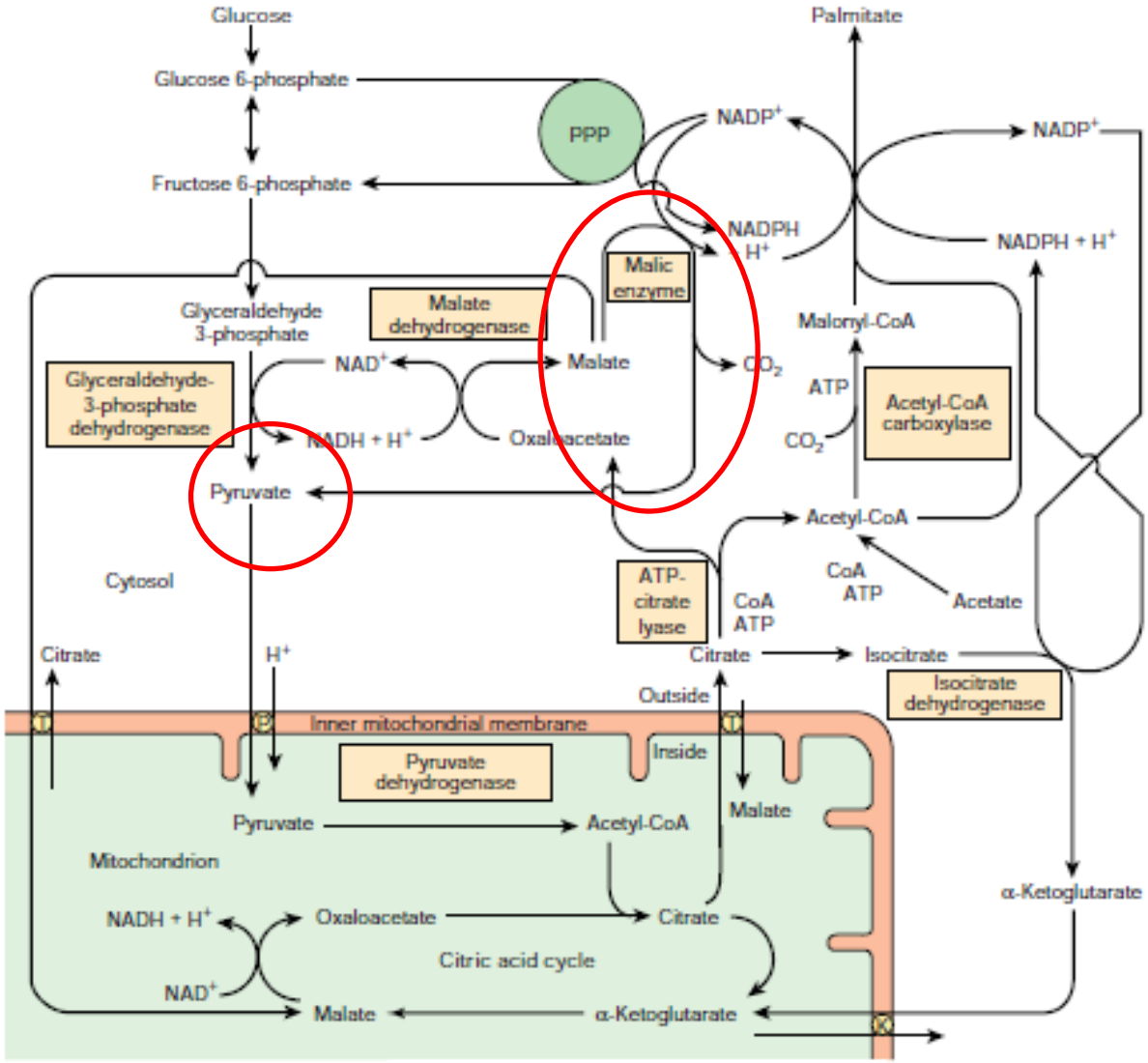
Enzyme: *ADP/ATP translocase*



Export of citrate



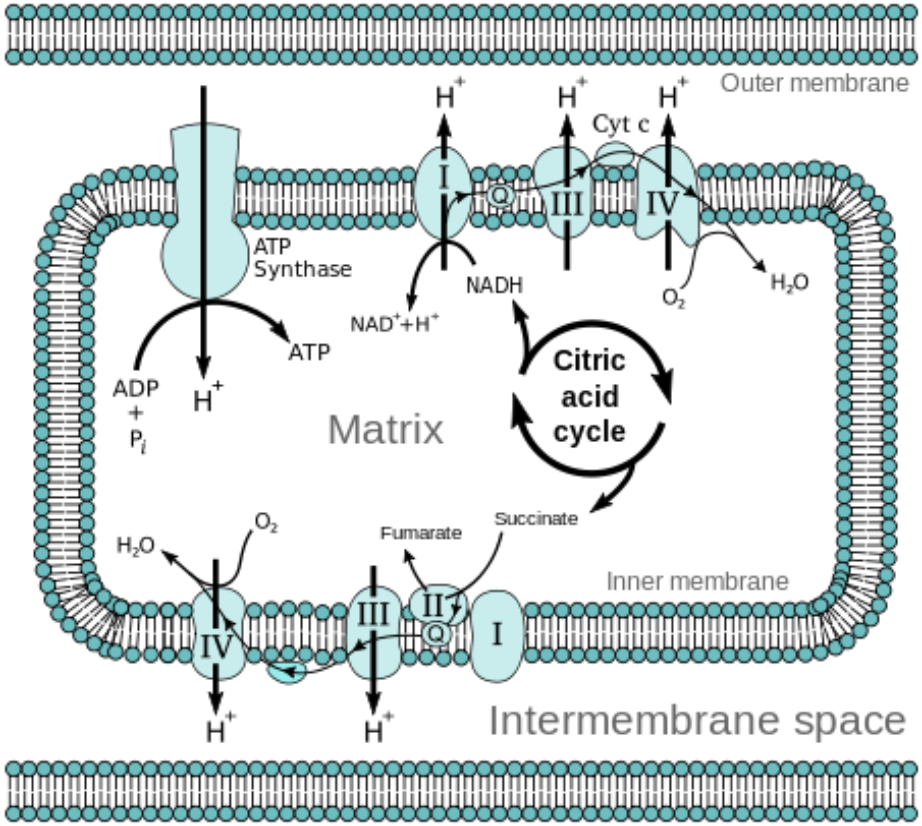
„Malic enzyme“



NADPH-malate dehydrogenase

= **source** of NAD**P**H except pentose phosphate pathway

the reaction that converts malate to pyruvate catalyzed by malic enzyme

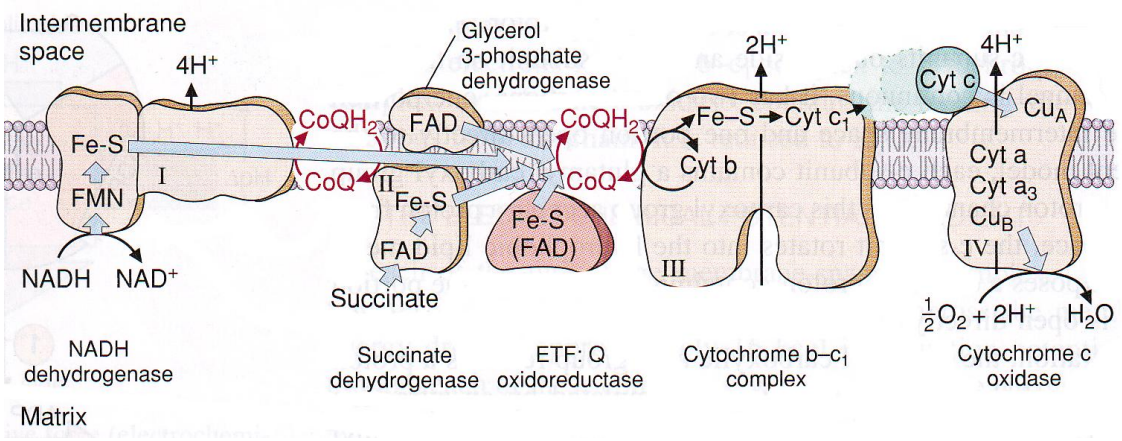


Summary I

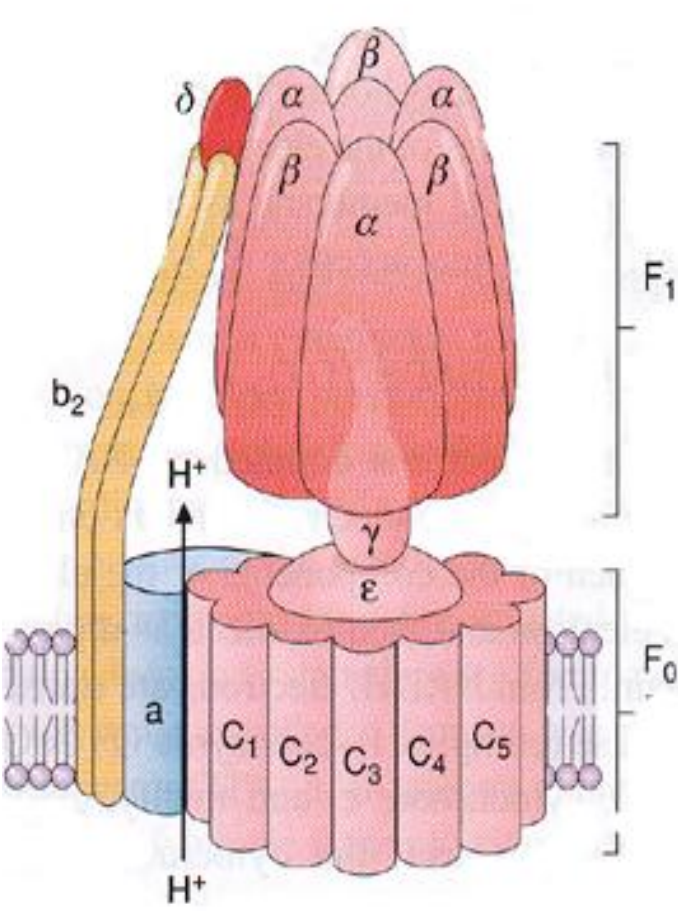
✓ **Respiratory chain (ETC)** is a sequence of redox reactions in direction of increasing potential, further **electrons are transferred to final acceptor oxygen.**

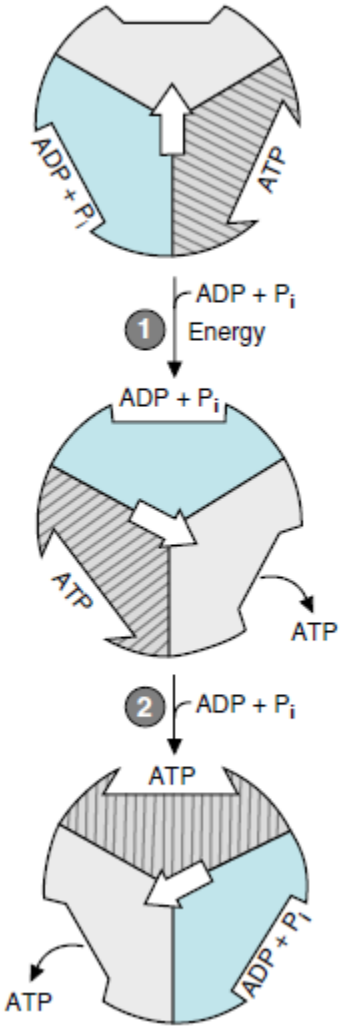
✓ Energy released in redox reaction is used for „**pumping**“ **H^+** from matrix into intermembrane space.

✓ **Production of proton-motive force.**



Summary II





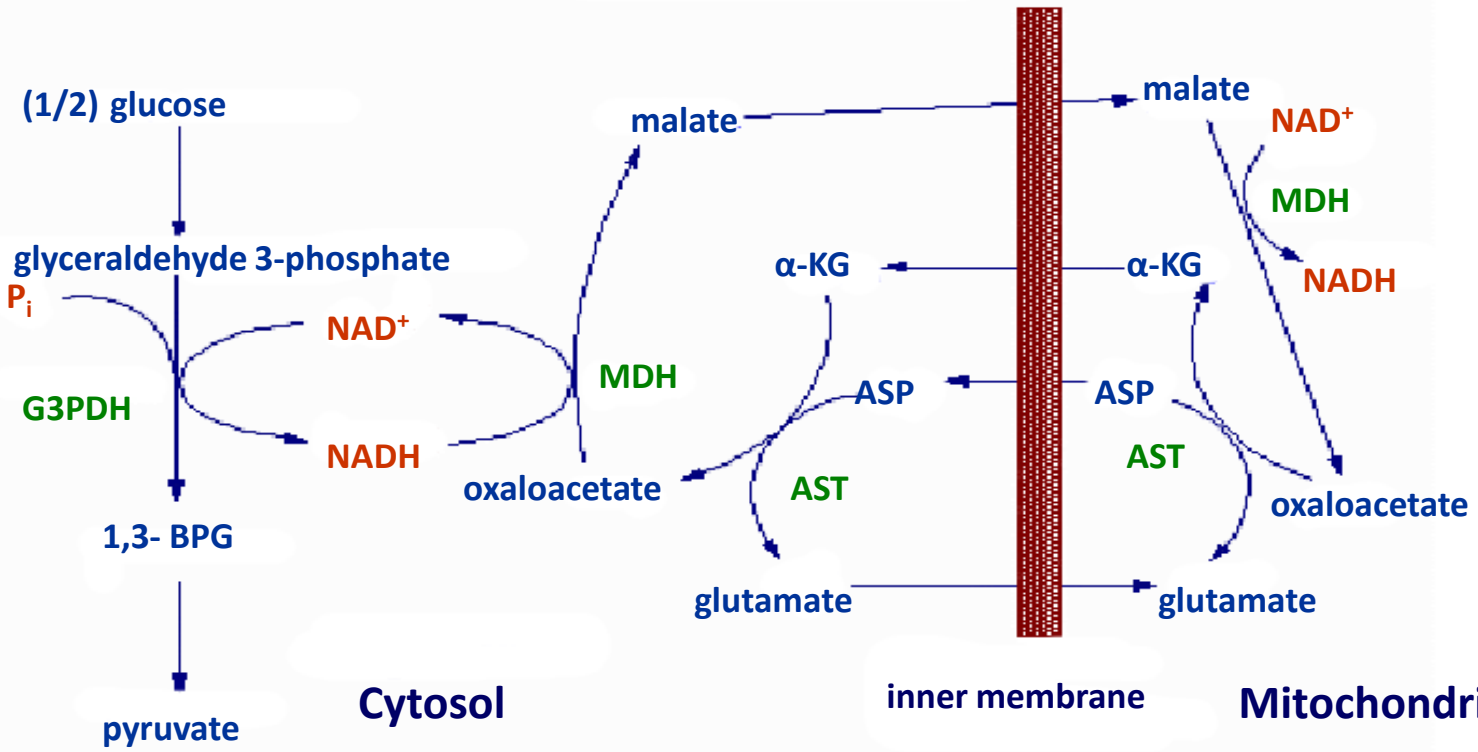
✓ Flow of H^+ back into matrix drives the **ATP synthesis** in process called **oxidative phosphorylation**.

✓ ATP synthesis takes place by changing the β-subunits conformation of ***ATP-synthase***.

✓ **Releasing** new synthetized **ATP** and **using for** biochemical reaction out of mitochondrion.

✓ **For transfer of ATP** the **specific transporter** *ADP/ATP translocase* is used.

Summary III



ASP – aspartate

α-KG – α-ketoglutarate

MDH – *malate dehydrogenase*

G3PDH – *glyceraldehyde-3-phosphate dehydrogenase*

AST – *aspartate aminotransferase*

✓ **Intermediates** of biochemical reactions **can be transferred** through the membrane **only** by using **transport systems**!

Literature

- D. Dobrota et all., **Lekárska biochémia**, first edition (2012), Osveta Publishing

- R. K. Murray et all., **Harper s Illustrated Biochemistry**, 28th edition (2009), The McGraw-Hill Companies, Inc.

- J. Koolman, K. H. Roehm, **Color Atlas of Biochemistry**, second edition (2005), Thieme

- M. Lieberman, A.D. Marks, **Marks´ Basic Medical Biochemistry A Clinical Approach**, third edition (2009)

- T. M. Devlin, **Textbook of Biochemistry with Clinical Correlations**, sixth edition (2006)

Thank you for your attention!

Time for your questions…

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